The Pathophysiologic Significance Of Endothelins In The Cerebral Circulation

Toshal R. Patel, B.Sc. (Hons.)

A thesis submitted for the degree of Doctor of Philosophy
to the Faculty of Medicine, University of Glasgow

Wellcome Surgical Institute &
Hugh Fraser Neuroscience Laboratories,
University of Glasgow,
Garscube Estate,
Bearsden Road,
Glasgow G61 1QH

© Toshal R. Patel, March 1996

ProQuest Number: 11007866

All rights reserved

INFORMATION TO ALL USERS
The quality of this reproduction is dependent upon the quality of the copy submitted.

In the unlikely event that the author did not send a complete manuscript
and there are missing pages, these will be noted. Also, if material had to be removed,
a note will indicate the deletion.

ProQuest 11007866

Published by ProQuest LLC (2018). Copyright of the Dissertation is held by the Author.

All rights reserved.
This work is protected against unauthorized copying under Title 17, United States Code
Microform Edition © ProQuest LLC.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

Thesis
10442
Copy 1

Table of Contents

Summary ---1

CHAPTER 1 INTRODUCTION

1.1 Anatomy of the cerebral circulation ---7

1.1.1 Cerebral circulation in man---7

1.1.2 The blood-brain barrier--8

1.1.3 Regulation of cerebrovascular tone------------- --------------------- ----9

Acetylcholine--10

Noradrenaline--11

5-Hydroxytryptamine (5-HT)---13

Dopamine--15

Histamine---16

Adenosine--17

Vasoactive Intestinal Polypeptide (VIP)-----------------------------------18

Neuropeptide Y (NPY)---19

Calcitonin Gene Related Peptide (CGRP)-------------------------------21

Substance P (SP)--22

Bradykinin---23

Angiotensin--23

Endothelium Derived Factors--26

Endothelium derived relaxing factor (EDRF)---------------------------26

Prostacyclin & Thromboxane A_2---28

1.2 Cerebrovascular disease--29

1.2.1 Cerebrovascular disease in man--29

Subtypes of ischaemic stroke--29

Thrombotic stroke--29

Embolic Stroke--30

Lacunar strokes---30

Haemorrhagic strokes--31

Intracerebral haemorrhage------31

Subarachnoid haemorrhage------33

Risk factors for stroke------33

1.2.2 Pathophysiology of cerebral ischaemia------35

Alterations in ion fluxes and energy metabolism------35

Mechanisms of Ischaemic Neuronal damage------38

Calcium------38

Excitatory Amino Acids------40

Free Radicals------42

The Ischaemic Penumbra------42

Cerebral Blood Flow and Cerebral Ischaemic Damage------43

1.2.3 Experimental models of cerebral ischaemia------44

Subdural Haematoma------46

Focal Ischaemia------47

Global Ischaemia------49

1.3 Endothelins a vasoconstrictor peptide------51

Discovery------51

Structure------52

Synthesis------52

Endothelin Receptors and Functions------53

Endothelin Receptors in Cerebral Vessels------55

Endothelin Receptors in the Central Nervous System------ 56

Endothelin Receptor Antagonists------58

Pathophysiological Role of Endothelins------59

Aims of Thesis------68

CHAPTER 2 MATERIALS AND METHODS

2.1 Rabbit Basilar Artery *in vitro*------72

Surgical Preparation------72

Experimental protocol------72

Statistical Analysis------73

Statistical Analysis--73

2.2 Measurement of calibre of feline pial arterioles *in situ* ---------------75

Surgical Preparation--75

Measurement of Arteriolar Calibre---76

Administration of Drugs---76

Statistical Analysis--78

2.3 Cerebral Blood Flow following Bilateral Common Carotid Artery Occlusion with Concomitant Haemorrhagic Hypotension in the Rat-------79

Surgical Preparation--79

Hydrogen electrode placement---79

Experimental Protocol--79

CBF measured by Hydrogen Clearance-------------------------------------80

CBF measured by [^{14}C]- Iodoantipyrine Autoradiography--------------80

Statistical Analysis--81

2.4 Cerebral Blood Flow following Bilateral Common Carotid Occlusion in the Rat--82

Surgical Preparation--82

Hydrogen electrode placement---82

Experimental Protocol--82

CBF measured by hydrogen clearance--------------------------------------82

CBF measured by [^{14}C]- Iodoantipyrine Autoradiography--------------82

Statistical Analysis--83

2.5 Cerebral Blood Flow following Subdural Haematoma in the Rat---84

Surgical Preparation--84

Experimental Protocol--84

CBF measured by [14C]- Iodoantipyrine Autoradiography---------------84

Statistical Analysis--85

2.6 Measurement of Pial Arteriolar Calibre following Permanent MCA Occlusion in the Cat---- --87

Surgical preparation--87

Measurement of Arteriolar Calibre----88
Administration of Drugs----88
Statistical Analysis----89

CHAPTER 3 **RESULTS**

3.1 Endothelin Receptors in the Cerebrovasculature----91

3.1.1 Rabbit Basilar Artery *in vitro*----91
Bosentan----91
BQ-123---- 91
ET-3----91
BQ-3020----92

3.1.2 Feline Cerebral Arterioles *in situ*----93
Perivascular Microapplication of BQ-123 *per se*----93
Perivascular co-application of ET-1 (10 nM) and BQ-123----93
Perivascular and intravascular administration of BQ-3020----93

3.2 Evaluation of Endothelin Receptor Antagonists in the Cerebrovasculature of the Cat----95

3.2.1 Cerebrovascular effects of Bosentan *in vivo*----95
Perivascular Microapplication of Bosentan----95
Perivascular co-Application of Bosentan and Endothelin-1----95
Reversal of ET-1 induced vasoconstrictions----96
Perivascular Microapplication of Endothelin 1 after Intravenous Administration of Bosentan----96

3.2.2 Cerebrovascular effects of PD155080 *in vivo*----97
Perivascular Microapplication of PD155080----97
Perivascular co-application of PD155080 and Endothelin-1----97
Reversal of ET-1 induced vasoconstrictions----97
Intravenous administration of PD155080----98

3.2.3 Cerebrovascular effects of PD145065 *in vivo*----99
Perivascular Microapplication of PD145065----99
Perivascular co-Application of PD145065 and Endothelin-1----99

Perivascular Microapplication of Endothelin 1 after Intravenous administration of PD145065------99

3.3 Pathophysiologic Significance of Endothelins in the Cerebral Circulation-------101

3.3.1 Cerebral Blood Flow following Bilateral Common Carotid Occlusion with Concomitant Haemorrhagic Hypotension in the Rat------101

Cardiovascular and Respiratory Variables------101

Hydrogen Clearance------102

Autoradiography------102

3.3.1 Cerebral Blood Flow following Bilateral Common Carotid Occlusion in the Rat------104

Cardiovascular and Respiratory Variables------104

Hydrogen Clearance------104

Autoradiography------105

3.3.3 Cerebral Blood Flow following Subdural Haematoma in the Rat-----106

Cardiovascular and Respiratory Variables------106

Effect of Bosentan on Cerebral Blood Flow following Subdural Haematoma------108

3.3.4 Alterations in Pial Arteriolar Calibre following Permanent MCA Occlusion in the Cat------109

Post MCA occlusion alterations in vascular diameter------109

Perivascular microapplication of Bosentan and CSF on arterioles following permanent MCA occlusion------110

Perivascular Microapplication of PD155080 and CSF on arterioles following permanent MCA Occlusion------111

CHAPTER 4 DISCUSSION

4.1 Endothelin Receptors And Endothelin Receptor Antagonists In The Cerebrovasculature------115

4.1.1 Endothelin Receptors------115

In vitro investigations--115

In vivo investigations--118

Endothelin Receptors in the Central Nervous System-------------------122

Concluding Remarks---125

Critical appraisal of *in vitro* and *in situ* techniques used for investigating responses of cerebral vessels---128

In vitro techniques--128

In situ techniques--131

4.1.2 Endothelin Receptor Antagonists--------------------------------------138

Concluding Remarks---143

4.2 Pathophysiological role of endothelin and endothelin receptor antagonists --144

4.2.1 Experimental Cerebrovascular Diseases---------------------------------- 145

Global Ischaemia--145

Subdural Haematoma---149

Subarachnoid Haemorrhage--153

Focal Ischaemia--155

Critical appraisal of techniques used for measuring cerebral blood flow------164

4.2.2 Sources and Triggers for endothelin synthesis following cerebrovascular injury---170

4.2.3 Pharmacotherapy of cerebral ischaemia comparisons with endothelin receptor antagonists---174

Focal Cerebral Ischaemia--174

Global Cerebral Ischaemia---178

4.3 Endothelins from gene to therapeutic agents------------------------------------180

REFERENCES---184

List of Tables   After Page No.

1. Neurotransmitters and peptides in the cerebral circulation------------------------10
2. *In vitro* selectivity of endothelin receptor antagonists and agonists---------------58
3. Putative roles of the endothelins in pathophysiology-----------------------------59
4. Potency of ET receptor agonists and antagonists in the rabbit basilar artery *in vitro*---91
5. Severe global cerebral ischaemia: Physiologic Variables (Hydrogen clearance study---107
6. Severe global cerebral ischaemia in the rat: Physiologic Variables (Autoradiography study)--107
7. Anatomical variations in CBF following severe global cerebral ischaemia in the rat: Autoradiography study---107
8. Moderate global cerebral ischaemia in the rat: Physiologic Variables (Hydrogen clearance study)---107
9. Anatomical variations in CBF following moderate global cerebral ischaemia in the rat: Autoradiography study---107
10. Subdural Haematoma in the rat: Physiologic Variables--------------------------107
11. Anatomical variations in CBF following subdural haematoma in the rat: Autoradiography study--107
12. Responses of isolated cerebral arteries to endothelins--------------------------115
13. Endothelin receptors in peripheral vascular and non-vascular tissues----------116
14. Responses of cerebral arteries and arterioles to endothelins- *in vivo* studies ---122
15. Endothelin receptor antagonists in global and focal cerebral ischaemia-------146
16. Endothelin receptor antagonists in subarachnoid haemorrhage----------------154
17. Triggers for the release of endothelins---170
18. Progression in endothelin research---180
19. Plasma/CSF endothelin-1 levels following subarachnoid haemorrhage in humans--181

List of Figures **After Page No.**

1. Schematic diagram of the cerebral circulation---------------------------------------8
2. Thresholds of cerebral blood flow results in alterations in neuronal function--35
3. Temporal evolution of neuronal damage: Relationship between level of CBF and Duration of ischaemia---36
4. Post-Ischaemic hypoperfusion---49
5. Structures of the endothelin peptides---52
6. Post-translational processing of prepro ET-1---------------------------------------53
7. Rabbit Basilar artery in vitro: Antagonism of ET-1 responses by BQ-123/Bosentan--91
8. Rabbit Basilar artery in vitro: Antagonism of ET-3 responses by BQ-123-----92
9. In situ vasomotor responses of cerebral arterioles to the adventitial application of BQ-123--93
10. In situ vasomotor responses of cerebral arterioles to the adventitial application of BQ-3020---93
11. In situ vasomotor responses of cerebral arterioles to the intracarotid infusion of BQ-3020---94
12. In situ vasomotor responses of cerebral arterioles to the adventitial application of Bosentan---95
13. Reversal of exogenous ET-1 constriction in vivo by Bosentan and PD155080 ---96
14. In situ vasomotor responses of cerebral arterioles following the intravenous administration of Bosentan--- 96
15. In situ vasomotor responses of cerebral arterioles to the adventitial application of PD155080--97
16. In situ vasomotor responses of cerebral arterioles following the intravenous administration of PD155080--98
17. In situ vasomotor responses of cerebral arterioles to the vasoconstrictor substances following the intravenous administration of PD155080-------------98

18. In situ vasomotor responses of cerebral arterioles to the adventitial application of PD145065--99

19. In situ vasomotor responses of cerebral arterioles following the intravenous administration of PD145065---99

20. Alterations in MABP following the intravenous ET-1 in the presence and absence of intravenous PD145065--107

21. MABP in severe global cerebral ischaemia in the rat-----------------------------107

22. CBF in the caudate nucleus and parietal cortex following severe global cerebral ischaemia: Hydrogen clearance study--107

23. Autoradiograms illustrating hypoperfusion in the caudate nucleus and sensory motor cortex following severe global cerebral ischaemia in the rat------------107

24. Alterations in CBF in cortical regions following severe global cerebral ischaemia in the rat: Autoradiography study--------------------------------------107

25. MABP in moderate global cerebral ischaemia in the rat------------------------107

26. CBF in the caudate nucleus and parietal cortex following moderate global cerebral ischaemia: Hydrogen clearance study-----------------------------------107

27. Alterations in CBF in cortical regions following severe global cerebral ischaemia in the rat: Autoradiography study--------------------------------------107

28. Autoradiograms illustrating the alteration in cerebral blood flow following subdural haematoma in the rat--107

29. Frequency distribution analysis of area/CBF in the ipsilateral and contralateral hemispheres following subdural haematoma in the rat: stereotaxic co-ordinates anterior 10.5 mm---107

30. Frequency distribution analysis of area/CBF in the ipsilateral and contralateral hemispheres following subdural haematoma in the rat: stereotaxic co-ordinates anterior 8.92 mm---107

31. Frequency distribution analysis of area/CBF in the ipsilateral and contralateral hemispheres following subdural haematoma in the rat: stereotaxic co-ordinates anterior 7.19 mm---107

32. Frequency distribution analysis of area/CBF in the ipsilateral and contralateral hemispheres following subdural haematoma in the rat: stereotaxic co-ordinates anterior 6.06 mm--107

33. Frequency distribution analysis of area/CBF in the ipsilateral and contralateral hemispheres following subdural haematoma in the rat: stereotaxic co-ordinates anterior 5.15 mm--107

34. Frequency distribution analysis of area/CBF in the ipsilateral and contralateral hemispheres following subdural haematoma in the rat: stereotaxic co-ordinates anterior 3.75 mm--107

35. Frequency distribution analysis of area/CBF in the ipsilateral and contralateral hemispheres following subdural haematoma in the rat: stereotaxic co-ordinates anterior 2.18 mm--107

36. Frequency distribution analysis of area/CBF in the ipsilateral and contralateral hemispheres following subdural haematoma in the rat: stereotaxic co-ordinates anterior 1.02 mm--107

37. Subdural haematoma in the rat: Volume of tissue perfused at pre-determined thresholds of CBF--107

38. Subdural haematoma in the rat brain swelling in the ipsilateral hemisphere--107

39. Vasomotor responses of cerebral arterioles following focal cerebral ischaemia in the cat: constricted arterioles---109

40. Vasomotor responses of cerebral arterioles following focal cerebral ischaemia in the cat: dilated arterioles--109

41. Time course of responses of individual arterioles following focal cerebral ischaemia in the cat: dilated pial arterioles---110

42. Time course of responses of individual arterioles following focal cerebral ischaemia in the cat: constricted pial arterioles------------------------------------110

43. Relationship between size of arteriolar examined and magnitude of response following adventitial application of ET-1 or CSF--------------------------------120

44. Schematic diagram speculating on the cerebrovascular accessibility of endothelin receptor antagonists--143

45. Effect of PD156707 on the volume of hemispheric neuronal; damage following focal cerebral ischaemia in the cat--161

46. Effect of PD156707 on laser doppler flow following focal cerebral ischaemia in the cat--- -------------161

47. Efficacy of neuroprotective agents in focal cerebral ischaemia in the cat----177

Acknowledgements

I would like to thank all the members of staff at the Wellcome Surgical Institute for their excellent help and generosity throughout the course of my Ph.D. My greatest thanks to Prof. James McCulloch for his expert guidance, encouragement and confidence throughout the three year period. I would also like to thank Prof. A. Murray Harper for his advice and help. I thank Dr. Campbell Wilson and Dr. Colin Smith for making me very welcome during my two stints in their labs at Zeneca Pharmaceuticals.

I am extremely grateful to Dr. Moira McAuley, Dr. Mhairi Macrae, Dr. Debbie Dewar, Dr. Karen Horsburgh and Gail Gartshore for all the help, guidance and varied discussions. I thank Elaine Irving, David Paterson, Ailsa McGregor and Mr. Michael Fitzpatrick for all their help in the proof reading of manuscripts and parts of this thesis. A special thanks to Dr. Kenny Mackay for putting up with a new student and helping me learn the ropes - the experience was invaluable and to Lyndsay Graham for her excellent secretarial skills and perseverance during the preparation of manuscripts.

The excellent assistance from all members of technical staff is gratefully acknowledged. I would like to thank Margaret Stewart and Gordon Littlejohn for entertaining most of the vague requests of a Ph.D. student. I would like to thank Joan Stewart, Michael Dunn, Lindsay Dover, Margaret Roberts, Hayley Dingwall and Marion Steele for all their help. I extremely grateful for the skill and expertise of the animal nurses, Christine, Morag and Gillian, during the conduct of the cat experiments.

Finally, I would like to thank my parents and grandparents for their unconditional encouragement and support despite the distances involved.

Preface And Declaration

This thesis presents results from investigations conducted in broadly defined areas:

(1) The examination of the functions of endothelin receptors in cerebral arteries and arterioles. The investigations were conducted using *in vitro* and *in situ* techniques in the rabbit and cat.

(2) The pharmacologic evaluation and validation of doses of endothelin receptor antagonists in the cerebrovasculature

(3) The assessment of the pathophysiologic role of the endothelins in experimental animal models of focal cerebral ischaemia, subdural haematoma and global cerebral ischaemia.

This thesis comprises my own original work and has not been presented previously as a thesis in any form. Some of the experiments reported in this thesis were carried out in collaboration with other investigators: Mr. S. Galbraith (focal cerebral ischaemia in the cat); Dr. C. Wilson and Dr. C.F.C. Smith at Zeneca Pharmaceuticals (*in vitro* investigations).

Summary

This thesis examined the pathophysiologic role of the endothelins in the cerebral circulation. Endothelin receptors were characterised in the rabbit basilar artery *in vitro* and in feline cerebral resistance arterioles *in situ*. Investigations in the feline cerebral resistance arterioles were designed to examine the cerebrovascular effects and blood-brain barrier penetration of non-peptide and peptide endothelin receptor antagonists. The pathophysiologic role of the endothelins were examined, using validated doses of endothelin receptor antagonists (primarily Bosentan), in experimental models of focal cerebral ischaemia in the cat, transient global cerebral ischaemia in the rat and subdural haematoma in the rat.

In the rabbit basilar artery *in vitro*, pre-incubation with either the combined ET_A/ET_B endothelin receptor antagonist Bosentan (4-tert- Butyl- N- [6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2, 2'- bipyrimidin- 4-yl]- benzene sulphonamide) or the endothelin ET_A receptor antagonist BQ-123 (cyclo D-Aspartate-D-Tryptophan-L-Leucine-D-Valine-L-Proline) had minimal effect on the resting tone of arterial segments. The ET_B receptor agonist BQ-3020 (N-Acetyl [11 Ala, 15 Ala] ET-1 (6-21)) elicited a small constriction of the arterial segments. Bosentan (10 μM) elicited a rightward shift of the ET-1 concentration response curve (pA_2 = 5.1). BQ-123 (0.1 - 10 μM) elicited a concentration dependent rightward shift of the ET-1 concentration response curve (pA_2 = 5.3). BQ-123 (1 μM) elicited a substantial rightward shift of the ET-3 concentration response curve (pA_2 = 7.2). The observations suggested the presence of an 'atypical' endothelin ET_A receptor mediating vasoconstriction in the rabbit basilar artery.

The receptors mediating the cerebrovascular actions of endothelins were examined in feline cerebral resistance arterioles *in vivo*. The adventitial microapplication of the endothelin ET_A receptor antagonist BQ-123 (0.1 - 10 μM) *per se* had minimal effect on cerebral resistance arterioles examined. The adventitial microapplication of endothelin-1 (10 nM) elicited a marked vasoconstriction of cerebral resistance arterioles (-29.1 ± 1.9 % from pre-injection baseline). The endothelin-1 induced vasoconstriction was attenuated, in a dose dependent manner, by

the adventitial co-application of BQ-123 and endothelin-1 (estimated IC_{50} 0.7 μM). The adventitial microapplication of the endothelin ET_B receptor agonist BQ-3020 (0.001 - 1 μM) effected a dose dependent vasodilatation (EC_{50} 30 nM, maximum response 25 ± 5 % from pre-injection baseline). The magnitude of the vasodilatation elicited by BQ-3020 (100 nM and 1 μM) was dependent on the pre-injection calibre of the arterioles examined. The intracarotid infusion (via the lingual artery) of BQ-3020 (0.5-500 pmol/min) had no significant effect on the calibre of cerebral resistance arterioles. These results suggest that the peptide endothelin ET_B receptor agonist fails to gain access to the cerebrovascular endothelin ET_B receptors following its intraluminal administration. These investigations indicate that endothelin ET_A receptors mediate vasoconstriction and endothelin ET_B receptors mediate vasodilatation in feline cerebral resistance arterioles *in vivo.*

The cerebrovascular actions of Bosentan, a novel endothelin antagonist with effects at ET_A and ET_B receptors, have been examined in individual pial arterioles on the cortical surface of chloralose-anaesthetised cats. Subarachnoid perivascular microapplication of Bosentan (0.3 - 300 μM) had minimal effect on pial arteriolar calibre. Subarachnoid perivascular microapplication of endothelin-1 (10 nM) effected a marked reduction in pial arteriolar calibre (reduced by 39.2 ± 2.7% from baseline). This vasomotor effect of topical endothelin could be attenuated either by co-administration of Bosentan (IC_{50} approximately 1 μM) or by the intravenous administration of Bosentan (17 μmol/kg). These investigations suggest that bioactive amounts of Bosentan (applied topically or systemically) gain access to the adventitial surface of cerebral arterioles and may be a valuable tool in the elucidation of the functional significance of endothelins in the cerebral circulation *in vivo.*

The cerebrovascular effects of the non-peptide endothelin receptor antagonist PD155080 (2-benzo (1,3) dioxol-5-yl-3-benzyl-4-(4-methoxyphenyl)-4-oxobut-2-enoate) and the hexapeptide endothelin receptor antagonist PD145065 (Acetyl-((R)-2(10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5-yl) Glycine -L-Leucine-L-Aspartate-L-Isoleucine-L-Isoleucine-L-Tryptophan) in feline cerebral resistance arterioles *in vivo* were contrasted with the effects of Bosentan. PD145065 (0.03 - 3 μM) and PD155080

(0.3 - 30 μM) per se had minimal effect on pial arteriolar calibre. The perivascular microapplication of ET-1 (10 nM) elicited a marked reduction in pial arteriolar calibre (-32 ± 2 % from pre-injection baseline). The perivascular co-application of PD145065 and ET-1 (10 nM) effected a dose dependent attenuation of the ET-1 vasoconstriction (IC_{50} 0.1 μM). The perivascular co-application of PD155080 and ET-1 (10 nM) effected a dose dependent attenuation of the ET-1 vasoconstriction (IC_{50} 1 μM). The intravenous administration of PD145065 (30 μmol/kg) had minimal effect on perivascular exogenous ET-1 induced vasoconstriction (pre-i.v. - 32 ± 2%; post i.v -28 ± 4%). However, intravenous administration of PD155080 (19.2 μmol/kg) markedly attenuated the ET-1 induced vasoconstriction (pre-i.v. - 32 ± 2%; post i.v 3 ± 4%). The dose of PD145065 (30 μmol/kg) used in the present investigations attenuated the pressor and depressor blood pressure responses elicited by an intravenous administration of endothelin-1 (1 nmol/kg). The comparison with Bosentan demonstrated the blood-brain barrier penetration of the small molecule non-peptide endothelin receptor antagonists and the poor blood-brain barrier penetration of the peptide endothelin receptor antagonist.

The role of endogenous endothelins in mediating post-ischaemic hypoperfusion following transient global ischaemia was investigated in halothane-anaesthetised rats. Pre-treatment with the broad spectrum (ET_A and ET_B) endothelin antagonist, Bosentan (17 μmol/kg) had minimal effect on post-ischaemic hypoperfusion, measured by hydrogen clearance, in the caudate nucleus and the parietal cortex in the 3 hours following bilateral common carotid artery occlusion with concomitant haemorrhagic hypotension (transient global ischaemia). In a separate series of rats with cerebral blood flow (CBF) measured by [^{14}C]- Iodoantipyrine autoradiography at 90 min. after carotid occlusion with concomitant haemorrhagic hypotension, Bosentan treatment failed to significantly alter CBF in any of the 35 brain regions examined. No significant alterations in CBF, measured by hydrogen clearance, were observed following transient bilateral common carotid artery occlusion. [^{14}C]-Iodoantipyrine autoradiography at 90 min. post-occlusion failed to demonstrate any significant increases in cerebral blood flow following transient

bilateral common carotid artery occlusion in any of the 35 brain regions examined in anaesthetised rats. The failure of the broad spectrum endothelin antagonist Bosentan, at concentrations known to inhibit the cerebrovascular effects of exogenous ET-1, provide no support for the view that endothelins have a major role in mediating acute post -ischaemic hypoperfusion.

The efficacy of Bosentan (17 μmol/kg) in altering cerebral blood flow was assessed following subdural haematoma in the rat. Cerebral blood flow was measured using [^{14}C]-Iodoantipyrine autoradiography 2.5 hours following the induction of the subdural haematoma. Marked reductions in cerebral blood flow were observed in cortex underlying the haematoma. Frequency distribution analysis of cerebral blood flow at 8 pre-selected coronal planes failed to demonstrate any significant effect by Bosentan. The volume of tissue perfused by cerebral blood flow >20 ml/100g/min was not altered by the pre-treatment with Bosentan (Vehicle 157 ± 42 mm^3; Bosentan 120 ± 24 mm^3). A marked swelling of the ipsilateral hemisphere was observed at 2.5 hours following the induction of the subdural haematoma and Bosentan did not alter the swelling (Vehicle 16 ± 5% of contralateral hemisphere; Bosentan 10 ± 2% of contralateral hemisphere). These results provide no support for a major role for the endothelins in the regulation of cerebral blood flow following subdural haematoma in the rat.

The actions of Bosentan and PD155080, non-peptide endothelin receptor antagonists, were examined in feline pial arterioles *in situ* following middle cerebral artery occlusion to gain insight into the cerebrovascular influence of endogenous endothelins in focal cerebral ischaemia. Immediately following permanent middle cerebral artery occlusion, all pial arterioles overlying the suprasylvian and ectosylvian gyri displayed marked dilatations which were maintained in a population of vessels but differentiated into sustained constrictions in others. Perivascular subarachnoid microinjections of Bosentan (30 μM), PD155080 (30 μM) and artificial cerebrospinal fluid, CSF (pH 7.2) were performed between 30 and 210 minutes following middle cerebral artery occlusion. The perivascular microapplication of Bosentan (30 μM) and PD155080 (30 μM) around pial vessels overlying the suprasylvian and ectosylvian

gyri, which are within the territory of the occluded middle cerebral artery, elicited an increase in the calibre of post-occlusion dilated and constricted pial arterioles. The perivascular microapplication of PD155080 (30 μM) around post-occlusion dilated pial arterioles overlying the ectosylvian and suprasylvian gyri elicited an increase in the calibre of arterioles (11 ± 2% from pre-injection baseline; n=36). The perivascular microapplication of Bosentan (30 μM) around post-occlusion dilated arterioles elicited an increase in the calibre of arterioles (16 ± 9% from pre-injection baseline; n=38). In contrast, the microapplication of CSF (pH 7.2) elicited small reductions in pial arteriolar calibre of post-occlusion dilated arterioles (-9 ± 1% from baseline; n=44). Perivascular microapplication of Bosentan or PD155080 had minimal effect upon the calibre of pial arterioles on the parasagittal gyrus (anterior cerebral artery territory) although these arterioles had also displayed sustained dilatation following middle cerebral artery occlusion. These investigations indicate that contractile factors (whose effects can be reversed with endothelin receptor antagonists) constrict or impair dilatation of cortical resistance arterioles in an acute cerebral ischaemic episode.

These investigations have provided a basis for further investigations of a role for the endothelins in cerebrovascular pathology and the therapeutic potential of endothelin receptor antagonists in the treatment of cerebrovascular diseases.

CHAPTER 1

INTRODUCTION

1.1 Anatomy of the cerebral circulation

1.1.1 Cerebral circulation in man

The survival of cerebral tissue is dependent upon the continuous delivery of substrates by the blood. The brain receives 20% of the total cardiac output and the absence of any stores of glucose/glycogen makes it imperative that blood supply to the brain is maintained especially under conditions of cerebral ischaemia .

The blood supply to the brain is derived from four main arteries, 2 common carotid arteries arising from the aortic arch and 2 vertebral arteries arising from the subclavian arteries. The internal carotid artery gives rise to three main branches: the anterior cerebral artery (ACA), middle cerebral artery (MCA) and anterior choroidal artery. The vertebral arteries fuse at the level of the medulla oblongata to form the basilar artery. The basilar artery bifurcates to form the posterior cerebral artery. On the ventral surface of the brain these inflow vessels are linked by communicating arteries to form the circle of Willis. Anastomotic links between the posterior cerebral arteries and the internal carotid arteries via the posterior communicating arteries complete the circle of Willis. The circle of Willis ensures that there is the equal distribution of blood to all regions of the brain via the anterior, middle and posterior cerebral arteries.

The anterior cerebral arteries are linked by the anterior communicating artery and pass in between the sagittal fissure and supply the frontal and medial aspects of the frontal and parietal lobes and the corpus callosum. The medial and striate branches of the anterior cerebral arteries supply deep subcortical structures such as the basal ganglia. The middle cerebral artery branches off the internal carotid artery. The MCA supplies most of the lateral aspect of the brain and branches of the MCA, e.g. the lenticulostriate branches are responsible for supplying the deep brain areas (caudate nucleus, globus pallidus and internal capsule). The anterior choroidal artery supplies the choroid plexus, hippocampal structures and posterior regions of the internal capsule.

The vertebro-basilar arterial system is responsible for supplying blood to the posterior regions of the cerebrum and parts of the cerebellum. The basilar artery in turn bifurcates to form two posterior cerebral arteries. The vertebral and basilar

arteries give rise to a number of branches, e.g., anterior spinal artery, posterior spinal artery, posterior inferior cerebellar artery, anterior inferior cerebellar artery and the superior cerebellar artery. These branches supply the medulla oblongata, ventral portions of the spinal cord, dorsal and ventral aspects of the cerebellum and the choroid plexus.

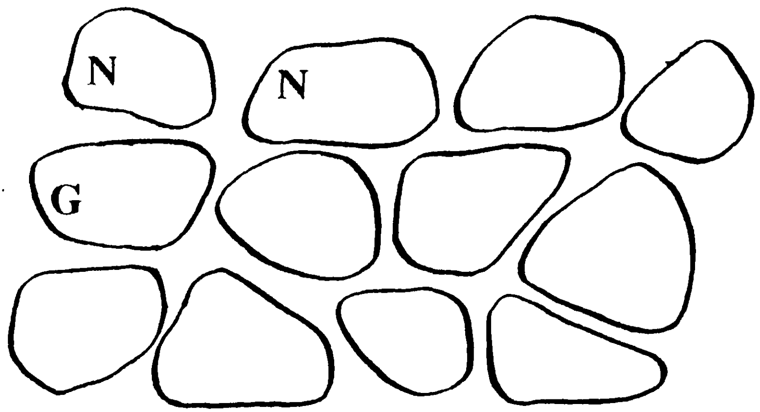
The posterior cerebral artery is responsible for the blood supply to the caudal regions of the cerebrum. Blood flow through the posterior cerebral artery (PCA) may be derived from the vertebro-basilar system or the internal carotid arteries via the posterior communicating arteries. The medial and lateral aspects of the temporal and occipital lobes are the main brain regions supplied by the posterior cerebral arteries. Branches of the posterior cerebral artery also supply the hippocampus and other subcortical structures. The posterior choroidal branch of the posterior cerebral artery supplies the choroid plexus.

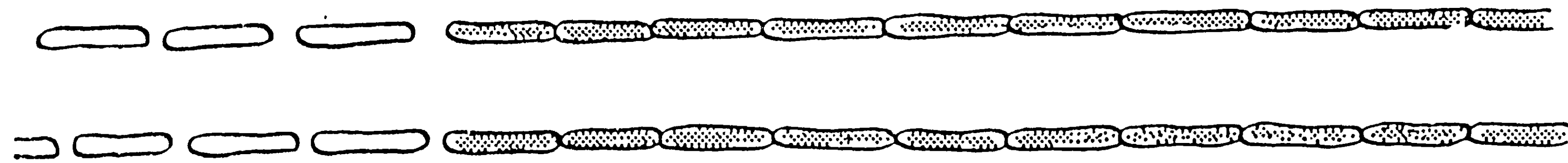
1.1.2 The blood-brain barrier

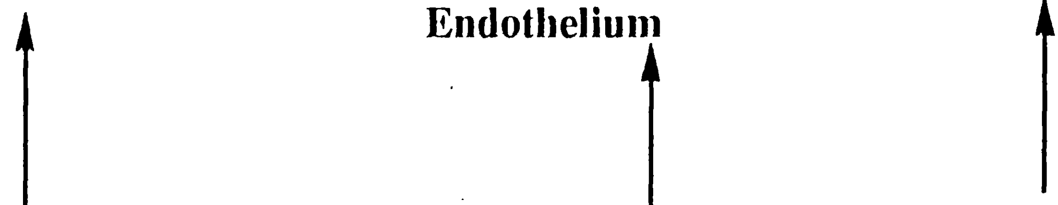
The blood-brain barrier consists of epithelial/endothelial cells that have tight junctions which prevent the passive movement of substances across the endothelium [Reese & Karnovsky, 1967]. There are specific transcellular transport mechanisms which facilitate the movement of solutes and substrates between the plasma and the interstitial fluid. There is an increased number of mitochondria for the energy dependent transporters that transport Na^+ and K^+ [Bradbury, 1979]. The absence of fenestrations and high electrical resistance of the cerebrovascular endothelium prevents access of substances to the brain tissue [Begley, 1992; Ermisch et al., 1993]. The cerebral capillaries consist of a continuous layer of endothelial cells connected by tight junctions [Brightman, 1992, Ermisch et al., 1993]. There is an absence of channels in the capillary wall that open to the interstitial fluid and the plasma. Endothelial cell membranes lack vesicular transport for the movement of substances from the plasma to the brain and *vice versa*. Damage to the blood-brain barrier may facilitate the entry of substances through the gaps that may be created between the endothelial cells (Figure 1).

Figure 1 Schematic representation of the cerebral circulation. The vascular endothelium constitutes the blood-brain barrier. The figure illustrates the gap junctions between endothelial cells at the level of the major cerebral arteries and the transition to tight junctions at the level of pial and intracerebral arterioles.

THE CEREBRAL CIRCULATION







Apart from the "physical " blood-brain barrier, there is increasing evidence for the existence of an enzymatic component to the blood-brain barrier [Hardebo & Owman, 1990]. The failure of some amine precursors such as L-DOPA and 5-Hydroxytryptophan to penetrate into the brain parenchyma is thought to be due to the degradation of these substances. Degradative enzymes such as L-Amino acid decarboxylase, monoamine oxidase (A & B), catechol-o-methyl transferase, butyryl cholinesterase and 4-Aminobutyrate aminotransferase have been identified in capillary and endothelial cells. Peptide transmitters and modulators in the peripheral circulation can be hydrolysed by peptidases in the plasma or those present on the surface of the cerebrovascular endothelium.

The cerebral endothelial cells are characterised by the tight junctions between them and the high electrical resistance which can prevent access of charged molecules to the brain. In recent years there has been an increasing shift to the development of *in vitro* models of the blood-brain barrier using endothelial cell cultures or co-cultures of cerebral endothelial cells and astrocytes [Laterra & Goldstein, 1992]. The examination of these *in vitro* models has indicated that the resistance of these cells is much lower than *in vivo* and may also affect the penetration of some substances that would normally be excluded.

1.1.3 Regulation of cerebrovascular tone

The pharmacological control of the cerebral circulation has been the area of much research. The "classical" view that the blood vessels of cerebral circulation were innervated by sympathetic and parasympathetic nerves similar to the peripheral vessels has recently undergone considerable modification. The role of the "classical' transmitters acetylcholine and noradrenaline in the control of CBF have been documented. However, more recent advances in molecular biology and immunocytochemical techniques has seen the identification of a variety of other transmitters in cerebral blood vessels. At the forefront of these discoveries have been the peptide molecules such as neuropeptide Y and substance P. Other important developments have seen the identification of the role of the vascular endothelium

derived factors such as nitric oxide and prostacyclin in the regulation of cerebrovascular tone and CBF. The following sections will try to highlight importance of the myriad of neurotransmitters and neuropeptides and their role in the regulation of cerebrovascular tone (Table 1).

Acetylcholine

Acetylcholine is perhaps the most studied of all the neurotransmitters. Its responses *in vivo* and *in vitro* have been well documented, low doses of acetylcholine induce vasodilatation while increasing the dose elicits a vasoconstriction [Edvinsson et al., 1977]. The responses are blocked in a reversible and competitive manner by atropine [Edvinsson et al., 1977]. The pre-constriction of cerebral vessels and the application of acetylcholine demonstrates an endothelium dependent vasodilatation, known to be mediated via EDRF/NO [Furchgott & Zawadski, 1980; Edvinsson et al., 1985c]. Transmural nerve stimulation of cerebral vessels also elicits a vasodilatation that is not attenuated by atropine or acetylcholinesterase inhibitors [Bevan et al., 1982; Duckles & Kennedy, 1982]. In the absence of the vascular endothelium acetylcholine elicits a vasoconstrictor response [Furchgott & Zawadski, 1980].

Muscarinic binding sites have been described in major cerebral arteries, pial arterioles and cerebral microvessels [Krause & Edvinsson, 1984; Tsukahara et al., 1986; Dauphin & MacKenzie, 1995 for review]. The microapplication or superfusion of acetylcholine or carbachol increases the calibre of feline pial arterioles *in situ* not dependent on the size of vessel examined [Kuschinsky et al., 1974; Haller, 1987]. The acetylcholine induced increases in pial arteriolar calibre were attenuated by atropine. The application of atropine *per se* to pial arterioles has minimal effect on the calibre of arterioles demonstrating minimal acetylcholine mediated vascular tone under resting conditions. Damage or removal of the vascular endothelium results in a loss of the dilator response *in vivo* and *in vitro* [Rosenblum et al., 1987; Haller et al., 1987]. Nitric oxide has been accepted as the mediator of this vasodilatation since the NOS inhibitors constrict the cerebral vessels *in vitro* and *in situ* and reduce CBF [Tanaka et al., 1991]. In some species, such as the pig the vasodilator substance is prostacyclin.

Table 1

Transmitters and Peptides regulating cerebrovascular tone

Transmitters	Response	Major Source
Acetylcholine	Dilatation	Nerve fibres from Sphenopalatine Ganglion
Noradrenaline	Constriction	Nerve fibres from Superior Cervical Ganglion
5-Hydroxytryptamine	Constriction	Nerve fibres from Raphe nuclei
Dopamine	Constriction/Dilatation	Nerve fibres
Histamine	Dilatation	Mast cells in perivascular spaces
Adenosine	Dilatation	Metabolism of nucleotide
Neuropeptides		
VIP	Dilatation	Nerve fibres from Sphenopalatine Ganglion
NPY	Constriction	Nerve fibres from Superior Cervical Ganglion
CGRP	Dilatation	Nerve fibres from Trigeminal Ganglion
Substance P	Dilatation	Nerve fibres from Trigeminal Ganglion
Bradykinin	Constriction	Neuronal
Angiotensin II	Constriction	
Endothelium derived factors		
EDRF/Nitric Oxide	Dilatation	
PGI_2	Dilatation	
Thromboxane A_2	Constriction	
Endothelins	Constriction	

Cholinergic innervation to the cerebral vessels predominantly arises from the sphenopalatine ganglion. Acetylcholinesterase containing neurones have been identified in the sphenopalatine ganglion and these may innervate the major cerebral arteries and the pial vessels [Dauphin & MacKenzie, 1995]. An intracerebral source of innervation has been suggested but, unlike noradrenaline or 5-Hydroxytryptamine, there is a diffuse distribution of cholinergic cell bodies suggesting that acetylcholine from this source may have a role in the local control of CBF [Dauphin & MacKenzie, 1995]. Nerves originating in the brainstem innervate the internal carotid artery and vessels of the circle of Willis (via the facial nerve and geniculate ganglion) [Dauphin & MacKenzie, 1995].

The stimulation of the sphenopalatine ganglion elicits an increase in CBF while ganglionectomy reduces the reactivity of the major cerebral arteries although the responses of the arterioles to hypoxia and hypercapnia are unaffected suggesting that although acetylcholine maybe present in neurones in the sphenopalatine ganglion, the increases in CBF elicited by nerve stimulation may not be directly related to the cholinergic innervation [Seylaz et al., 1988; Dauphin & MacKenzie et al., 1995]. The actions of acetylcholine may be mediated via an indirect mechanism where acetylcholine inhibits the release of vasoconstrictor agents [Edvinsson et al., 1977].

The intracarotid infusion of acetylcholine increases CBF in a variety of species including canine, feline, rat and rabbit [Alborch, 1981; De Ley et al., 1984; Dauphin & MacKenzie 1995]. Atropine attenuates the reduction in CBF while inhibition of acetylcholinesterase by physostigmine increases CBF and this latter response is also sensitive to atropine [Trigeuro et al., 1988; Dauphin et al., 1991].

Noradrenaline

Plexuses of noradrenaline containing nerve fibres, arising from the superior cervical ganglion, can be found around cerebral arteries, arterioles and veins [MacKenzie & Scatton, 1987]. There is a sparse contribution from the stellate ganglion but caudal portions of the circle of Willis are supplied from this source. The presence of noradrenaline has been identified in cortical pial vessels and cerebral

microvessels and higher levels of noradrenaline have been found in the larger cerebral arteries than the smaller pial arterioles [Duverger et al., 1987]. There is an increased uptake of noradrenaline in the cerebral arteries where there is greater noradrenergic innervation (e.g. ACA, MCA, PCA and the superior cerebellar artery) [MacKenzie & Scatton, 1987]. The available evidence indicates that there is an increased release from nerves in cerebral arteries than in peripheral arteries.

The transmural stimulation of the noradrenergic innervation elicits a species dependent response in cerebral arteries [MacKenzie & Scatton, 1987]. In the cat, transmural nerve stimulation elicited a dilatation of the cerebral arteries but the response is not cholinergic in origin and persisted after the administration of adrenergic antagonists, reserpine and chronic sympathectomy [Lee et al., 1978]. Further investigations have indicated that the response of feline arteries may be due to the release of vasodilator agents that may be co-localised with noradrenaline [MacKenzie & Scatton, 1987]. In other species, the constrictor response is the predominant response [MacKenzie & Scatton, 1987]. The constriction/dilatation of the cerebral arteries, is thus dependent on the species used and the localisation of the artery/arterioles examined. The effects of nerve stimulation are further complicated by the localisation of nerve fibres of different transmitters in the perivascular space. The differences in these responses are probably due to the co-transmitters such as NPY that are also released by nerve stimulation.

In vitro studies have suggested that α-adrenoceptors mediate constrictions in feline/canine vessels [Skarby et al., 1983]. The contractile responses to noradrenaline can be potentiated by the removal of the endothelium. The contractile responses to noradrenaline are calcium dependent [MacKenzie & Scatton, 1987]. Existence of ß-adrenoceptor mediated vasodilatation has been demonstrated in pre-contracted cerebral arteries *in vitro* [Edvinsson & Owman, 1974; Winquist et al., 1982]. Presynaptic localisation of α_2 adrenoceptors have been identified in cerebral arteries and these may inhibit the release of noradrenaline [Duckles, 1982]. Co-localisation of noradrenaline with neuropeptide Y has been identified in some vessels (see section on NPY).

In vivo stimulation of the superior cervical ganglion in the feline resulted in the constriction of pial arterioles (only those >100 μm) while there was no effect on the smaller arterioles <100 μm [Kuschinsky & Wahl, 1975; Auer et al., 1981 & 1983]. The effects were attenuated by a-adrenoceptor antagonists such as phentolamine [Auer et al., 1983]. The perivascular microapplication of noradrenaline constricts pial arterioles *in situ*, although in contrast to the stimulation of the superior cervical ganglion there was no dependence of the response on the calibre of arterioles [Kuschinsky & Wahl, 1975; Edvinsson, 1982].

The catecholamines elicit a minor reduction in CBF but these reductions have been observed in species where there is a contribution from extracranial circulation e.g. in the dog and the goat [MacKenzie & Scatton, 1987]. Other authors have reported a minimal alteration probably due to the inaccessibility of the amine to the cerebrovasculature [See section on the Blood-Brain Barrier]. The use of intravascular noradrenaline resulted in an increase in mean arterial blood pressure and if the increase was greater than the upper limit of the autoregulation, there would be an increase in CBF. The intraventricular administration of noradrenaline resulted in an increase in the glucose use, oxygen consumption and CBF. The noradrenaline induced increases in CBF may be related to the increased metabolic demand.

Some reports have suggested that noradrenaline may alter blood-brain barrier permeability. The stimulation of extrinsic sympathetic nerves can increase the permeability of capillaries. Monoamine oxidase has been identified in the endothelial cells and MAO inhibitors may facilitate the access of the monoamine oxidase to the cerebrovasculature.

5-Hydroxytryptamine (5-HT)

5-HT is a potent constrictor of the vasculature but the presence of the blood-brain barrier and the systemic effects of 5-HT complicate the interpretation of the effects of 5-HT on the cerebral circulation [See section on Blood-Brain barrier]. 5-HT innervation in the cerebral circulation originates from the Raphe nuclei in the brain stem [MacKenzie & Scatton, 1987]. In animals with an intact blood-brain barrier, 5-

HT has minimal effect on CBF [Harper & MacKenzie, 1977; Mendelow et al., 1977]. In situations where the blood-brain barrier is compromised, 5-HT decreased CBF and the reductions were confined to these areas. The absence of an effect of 5-HT when the blood-brain barrier is intact either due to the failure of the amine to directly permeate the blood-brain barrier or the inactivation of 5-HT (see section on the Blood-Brain Barrier).

5-HT induces marked constriction of cerebral arteries *in vitro* [MacKenzie & Scatton, 1987]. The cerebral arteries appear to be more sensitive to 5-HT than peripheral arteries. The 5-HT induced contractions are calcium sensitive. 5-HT has been demonstrated to dilate pre-constricted cerebral arteries and the responses were attenuated by ß-adrenoceptor antagonists [Edvinsson & Owman, 1974]. The microapplication of 5-HT on cortical pial arterioles *in situ* elicited a vasodilatation which was greater in arterioles <70 μm and a constriction in arterioles >150 μm [Harper & MacKenzie, 1977]. The administration of 5-HT into the brain reduces the EEG activity and reduces oxygen consumption and the reductions in CBF may be consequent to the metabolic depression [Harper & MacKenzie, 1977; Grome & Harper, 1983]. Administration of 5-HT directly into the brain or lesioning of 5-HT pathways results in a reduction in metabolism and a moderate reduction in CBF. Stimulation of the dorsal or medial Raphe nuclei increases glucose use and CBF but the increases in CBF are not related to alterations in glucose use. In contrast, in anaesthetised animals the stimulation of the Raphe nuclei reduces CBF and these effects may be related to the anaesthetic mediated metabolic depression [Bonvento et al., 1989].

Monoamine oxidase present in the cerebral endothelial cells will prevent access of 5-HT to the cerebrovasculature. However, high concentrations of 5-HT have been found in endothelial cells and a role for 5-HT in the regulation of blood-brain barrier permeability has been postulated [Mackenzie & Scatton, 1987].

Dopamine

Dopamine is a potent contractile agent of cerebral arteries *in vitro* and the maximum contractions elicited by dopamine are greater than those elicited by noradrenaline [Hamel et al., 1988]. The contractile actions may be mediated via a-adrenoceptors or 5-HT receptors as well as dopamine receptors [Edvinsson et al., 1993]. Dopamine agonists such as apomorphine have some contractile effects on cerebral arteries but the effects are less pronounced than those of dopamine.

Dopamine and dopamine agonists have been reported to induce dilatation of major cerebral arteries *in vitro* in many species (man, cat, dog, rabbit, rat) [Edvinsson et al., 1993]. The demonstration of the relaxant effects of dopamine often requires the pre-contraction of the vessels, although in some species the relaxant effects can be elicited in the absence of pre-constriction. The signal transduction mechanisms associated with dopamine receptors is unclear but indication are that the dilator responses are mediated by the activation of adenylate cyclase and release of cAMP [Amenta et al., 1984].

The intracarotid infusion of dopamine constricts cerebral arteries (basilar artery) and cortical surface arterioles *in situ* [Edvinsson et al., 1985b]. Dopamine receptor antagonists have minor contractile effects when compared to dopamine. Dopamine D_1 agonists such as apomorphine, SKF38393 and LY141865 have vasodilator actions on pial arterioles *in situ* [Edvinsson et al., 1985b]. These responses are attenuated by the D_1 antagonists SCH23390 [Edvinsson et al, 1985b]. Despite the demonstration of vasodilatation by dopamine agonists the perivascular microapplication of dopamine fails to demonstrate any vasodilator response [Edvinsson et al., 1985b]. However, the superfusion of dopamine on the cortical surface arterioles but the increases may be the result of an increase in oxidative metabolism in the underlying cerebral cortex [Altura et al., 1980].

Dopamine is a transmitter in the CNS and activation of dopamine receptors can alter cerebral function. Dopamine agonists such as apomorphine and amphetamines increase the cerebral metabolic rate of oxygen consumption ($CMRO_2$) and the cerebral metabolic rate of glucose use (CMR_{glc}) [McCulloch et al., 1982b]. In contrast, the

dopamine antagonists *per se* have minimal effect on cerebral metabolism [McCulloch et al., 1982b]. Direct or indirect releasers of dopamine such as the amphetamines or phenylethylamine increase CBF [Edvinsson et al., 1993]. The increases in CBF are accompanied by increases in oxidative metabolism and the responses are attenuated by dopamine receptor antagonists [McCulloch et al., 1982b]. The mechanism of increases in CBF elicited by dopamine agonists such as apomorphine and bromocriptine is unclear but increases in CBF may be the result of an increase in the oxidative metabolism elicited by these agents [Bes et al., 1982; Leenders et al., 1985]. The absence of any effect of dopamine antagonists on CBF suggests that dopamine may play a minor role in the regulation of basal CBF.

Histamine

Histamine is associated with the cerebrovasculature and brain tissue. Mast cells are thought to be the major source of histamine in the cerebrovasculature and have been localised in the perivascular spaces of the dura, pial vessels, choroid plexus and in the cerebral microvessels [Edvinsson & MacKenzie, 1977]. The responses of cerebral vessels to histamine is species dependent. In some species, such as the feline and guinea pig, histamine constricts cerebral vessels while in other species (canine, rat) histamine elicits a vasodilator response [Ottosson et al., 1988; Toda et al., 1990]. The general consensus is that histamine H_2 receptors mediate the vasodilator responses while the constrictor responses are mediated by H_1 receptors [Edvinsson & Owman, 1975; Ottosson et al., 1990]. Although there is emerging evidence that the responses of cerebral vessels to histamine may be dependent on species and the segment of the vessel examined [Edvinsson et al., 1993]. Vasodilatation elicited by histamine is associated with a depolarisation of the vascular smooth muscle while the removal of the vascular endothelium attenuates the histamine mediated vasodilatation [Chang et al., 1988; Toda et al., 1990].

The perivascular administration of histamine in feline cerebral arterioles *in situ* elicits a vasodilatation mediated via H_2 receptors [Wahl & Kuschinsky, 1979; Gross et al., 1981a]. Superfusion on the cortical surface in the feline elicits a vasodilatation

while in the mouse and rabbit there is a vasoconstriction [Raper et al., 1972; Kamitani et al., 1985]. There are suggestions that in the latter species histamine may be acting to release an endothelium derived contracting factor. There is a greater abundance of H_2 receptors on arterioles and evidence suggests that H_2 receptors may be more sensitive to histamine and only small circulating concentrations may be required to elicit the vasodilator response.

The intravascular administration of histamine does not affect CBF due to its poor blood-brain barrier permeability [Oldendorf et al., 1971]. Some authors have reported that histamine can reduce CBF. The application of histamine by circumventing the blood-brain barrier elicits increases in CBF but the concentrations required are almost 100 fold greater than the concentrations required in the microapplication studies [Gross et al., 1981a].

In the peripheral circulation, histamine increases vascular permeability and causes oedema [Gross et al., 1981b]. Histamine may have a similar effect on the cerebral circulation. In cerebral capillaries, a histamine sensitive adenylate cyclase mechanism that may affect blood-brain barrier transport has been described [Karnushina et al., 1980]. High concentrations of histamine (100 μM) can cause an increased permeability of the blood-brain barrier to sucrose and α-aminoisobutyric acid [Dux & Joo, 1982; Gross et al., 1981b]. Superfusion with histamine increases the permeability of fluorescein dye into the brain parenchyma [Wahl et al., 1988]. Additionally, histamine has been demonstrated to stimulate pinocytosis in cerebrovascular endothelial cells [Karnushina et al., 1980; Sercombe et al., 1986]. The significance of these mechanisms to the regulation of CBF and in cerebrovascular pathology is unknown.

Adenosine

Adenosine dilates pial arterioles in a dose dependent manner and the responses are attenuated by theophylline. There are some suggestions that adenosine increases the calibre of arterioles under normocapnic conditions more than under hypoxic or hypercapnic conditions [Gregory et al., 1980]. The adenosine responses are mediated

by A_2 receptors with the cerebral veins being less reactive than arteries or arterioles [Ibayashi et al., 1991]. There is no correlation between the vessel size and the response to adenosine [Kuschinsky & Wahl, 1976]. There are species differences in the responses of cerebral arteries and arterioles to adenosine.

Inhibition of the uptake of adenosine by agents such as dipyridamole, reduces the vasoconstriction (including response to hypocapnia). The response is probably due to an increase in the endogenous levels of adenosine as a result of the inhibition of uptake [Ibayashi et al., 1988]. Adenosine may be involved in the coupling of CBF to neuronal activity and glucose use [Ibayashi et al., 1991]. However, the adenosine antagonists such as theophylline and uptake inhibitor dipyridamole have minimal effect on feline cerebral arteriolar calibre suggesting a minimal role for adenosine in the regulation of vascular tone [Kuschinsky & Wahl, 1976].

Elevations in endogenous adenosine levels have been measured following ischaemia, hypotension, hypoxia and seizures [Morii et al., 1984; Winn et al., 1985]. The alterations in adenosine levels, measured by microdialysis parallel the increases in CBF observed in seizures [Park et al., 1987; Phillis et al., 1987]. The increases in endogenous adenosine levels suggests that these conditions may cause a sufficient elevation in adenosine levels to increase CBF.

The effects of exogenous adenosine on CBF are more varied. Some authors have reported no alterations following the intravascular administration while others have reported increases in CBF in rabbits, primates and man [Edvinsson et al., 1993]. Adenosine may not penetrate the blood-brain barrier but a nucleoside transporter may facilitate the entry into the brain [Ermisch et al., 1993]. The alterations elicited by adenosine are rapid and one cannot discount an endothelium dependent mechanism. The uptake inhibitor dipyridamole increases CBF and this may be due to increased endogenous levels of adenosine [Phillis et al., 1990].

Vasoactive Intestinal Polypeptide (VIP)

VIP is a vasodilator of cerebral vessels *in vitro* and *in situ*. The direct administration of VIP into the brain, circumventing the blood-brain barrier, induces an

increase in CBF [Rorstad et al., 1993; Dauphin & MacKenzie, 1995]. A related peptide Peptide Histidine Isoleucine (PHI) also elicits a concentration dependent dilatation of cerebral blood vessels [Edvinsson & McCulloch, 1985] and is approximately 25-60 fold less potent than VIP in eliciting vasodilatation [McCulloch & Edvinsson, 1980; Edvinsson & McCulloch, 1985]. The investigations have demonstrated that VIP is less active in producing vasodilatation in cerebral veins than in cerebral arterioles [McCulloch & Edvinsson, 1980; Edvinsson & McCulloch, 1985]. The magnitude of the response of VIP on cerebral arterioles is dependent on the calibre of arterioles examined with cerebral arterioles <70μm demonstrating an increased reactivity to VIP [McCulloch & Edvinsson, 1980]. The vasodilatation induced by VIP is not endothelium dependent and VIP/PHI activate adenylate cyclase and increase levels of cAMP in vascular smooth muscle [Edvinsson et al., 1985a]. There is evidence for the co-localisation of VIP in cholinergic neurones but the role of acetylcholine/VIP interactions in the cerebrovasculature is unclear [Dauphin & MacKenzie, 1995].

VIP/PHI have been implicated as neurotransmitters in the peripheral and central nervous systems [Rorstad et al., 1993; Rostene, 1984]. PHI like peptide has been identified in the rat brain and the release of PHI is calcium dependent indicating release from a transmitter pool [Korchak et al., 1985; Dauphin & MacKenzie, 1995]. VIP/PHI have been localised in neurones in the neocortex, amygdala and hippocampus [Benfield et al., 1984]. The intrastriatal administration of VIP resulted in heterogeneous alterations in glucose use in the caudate nucleus and increased CBF [McCulloch et al., 1983].

Neuropeptide Y (NPY)

NPY is found in cerebrovascular sympathetic nerves and is a potent constrictor of cerebral arteries, arterioles and veins in all species examined [Edvinsson et al., 1983; Edvinsson et al., 1985b; Edvinsson et al., 1987; Meija et al., 1988]. NPY elicits vasoconstrictor action of longer duration than that elicited by noradrenaline in cerebral vessels ($t_{1/2}$ = 5 min) [Edvinsson et al., 1984]. There is evidence that NPY

may be co-localised with noradrenaline in cerebrovascular sympathetic nerves and NPY may serve to potentiate the actions of noradrenaline [Abel & Han, 1989]. The vasoconstrictor actions of NPY are calcium dependent and can be attenuated by the calcium channel antagonists. Despite the co-localisation of NPY with noradrenaline, adrenoceptor blockade does not attenuate the vasoconstrictor action of NPY [Edvinsson et al., 1983]. The vasoconstrictor actions of NPY are mediated via Y1 receptors present on the cerebrovascular smooth muscle. The actions of NPY are modulated by receptor subtypes Y_1 and Y_2 [Wahlestedt et al., 1985]. The Y_1 receptors are located on cerebrovascular smooth muscle. In the periphery, NPY can act directly on postsynaptic receptors to induce vasoconstriction, can act postsynaptically to amplify vascular constriction elicited by other transmitters or can induce vasodilatation by the presynaptic modulation of noradrenaline release [Wahlestedt et al., 1985; Westfall et al., 1987]. The effects of NPY are not dependent on an intact vascular endothelium, however, an attenuation of the actions of NPY following the removal of the endothelium have been reported [Daly & Hieble, 1987]. At the intracellular level, NPY induces a reduction in cAMP accumulation in feline cerebral vessels while in the guinea pig basilar artery the vasoconstriction elicited by NPY is accompanied by a slow depolarisation of vascular smooth muscle [Fallgren et al., 1990].

The actions of NPY may involve the modulation of vasodilatation elicited by acetylcholine, adenosine, noradrenaline or substance P [Han & Abel, 1987; Fallgren et al., 1989]. NPY does not have any effect on the vasodilatation elicited by VIP or calcitonin gene related peptide (CGRP) [Fallgren et al., 1989]. In the periphery, NPY has been shown to inhibit the release of noradrenaline from sympathetic nerve fibres [Westfall et al., 1987; Linton-Dahlof & Dahlof, 1993]. In peripheral tissues NPY reduces the contractile responses induced by transmural nerve stimulation. Presynaptic modulation of adrenergic mechanisms can effect NPY release but the mechanism is unclear.

There have been reports on the effects of NPY administration on CBF and glucose use [Tuor et al., 1990]. The *in vitro* and *in situ* investigations have suggested

that NPY should reduce CBF. Allen and colleagues (1985) reported that the intracarotid injection of NPY reduced CBF in the rat cortex for up to 2 hours and other investigations have demonstrated a dose dependent reduction in striatal CBF [Tuor et al., 1985]. NPY has minimal effect on mean arterial blood pressure.

Calcitonin Gene Related Peptide (CGRP)

CGRP is a 37 amino acid peptide that is released from the trigeminal nerves. CGRP immunoreactivity and binding sites have been identified in the CNS, mainly in the hypothalamus, amygdala and brainstem [Amara et al., 1982; Rosenfeld et al., 1983; Skofitsch & Jacobowitz, 1985]. CGRP in often co-localised with SP in the trigeminal ganglion but there are a greater number CGRP containing cell bodies in the ganglion [Uddmann et al., 1985; McCulloch et al., 1986; Gulbenkian & Wharton, 1993]. CGRP containing neurones arise from various sub-divisions of the trigeminal system and in turn innervate blood vessels of the circle of Willis, extracranial and intracranial portions of the internal carotid arteries and the vertebro-basilar system [Edvinsson et al., 1989; Saito & Moskowitz, 1989]. CGRP containing varicosities have been observed in the adventitia and medial/adventitial border of the MCA, ACA, PCA , vertebral arteries and pial arterioles [McCulloch et al., 1986].

CGRP elicits vasodilatation of cerebral arterioles in all species examined [McCulloch & Edvinsson, 1987; McCulloch et al., 1986; Jansen et al., 1993]. CGRP is a more potent dilator than SP and elicits vasodilatations of a greater magnitude. The vasodilatation is not endothelium dependent suggesting an absence for a role for nitric oxide [Edvinsson et al., 1985]. The vasodilator response elicited by CGRP is maintained for minutes and suggests a role for CGRP in the restoration of vascular diameter following vasoconstriction in an attempt to maintain CBF [McCulloch et al., 1986]. Lesioning of the trigeminal system has no effect on the vasodilator capacity of cerebral arterioles but it prolongs the vasoconstriction induced by noradrenaline and $PGF_{2\alpha}$ [McCulloch et al., 1986; McCulloch & Edvinsson, 1987].

Substance P (SP)

SP has been identified in nerve fibres arising from the trigeminal ganglion and innervating cerebral arteries, pial arterioles and veins in the choroid plexus and the dura [Edvinsson et al., 1981; Suzuki et al., 1989]. SP neurones arising from the brain stem may innervate arterioles and capillaries in the brain stem [Kapadia & Lanerolle, 1984]. SP neurones arising from the dorsal root ganglia innervate the caudal portions of cerebral vessels [Edvinsson et al., 1981; Saito & Moskowitz, 1989].

Increases in SP release has been demonstrated with electrical field stimulation and capsaicin or K+ evoked depolarisation [Moskowitz et al., 1983]. The release of SP from neurones is a calcium dependent process [Moskowitz et al., 1983]. SP induces a dose dependent dilatation of cerebrovascular smooth muscle in a variety of species, although, there is evidence that the responses are highly variable with some authors reporting no alterations with SP [Edvinsson et al., 1981; Jansen et al., 1991].

The vasodilatation of SP is endothelium dependent and the mediator of this vasodilatation is unclear but SP has been reported to increase levels of cGMP [Rapoport & Murad, 1983; Edvinsson & Jansen, 1987]. The microapplication of SP on cerebral arterioles *in situ* elicited a concentration dependent dilatation [Edvinsson et al., 1982]. The indications were that the arterioles were more sensitive than veins to SP application but there was no correlation between the arteriolar calibre and the magnitude of the dilatation elicited [Edvinsson et al., 1981 & 1982]. The intracerebral administration of SP increased blood flow in the hypothalamus, however, these elevations were attenuated by cholinergic antagonists, sympathectomy and adrenoceptor antagonism [Klugman et al., 1980]. Another important aspect of the pharmacology of SP is its ability to increase vascular permeability of blood vessels in the periphery [Lembeck & Gamse, 1982; Regoli et al., 1993]. There is limited evidence in the cerebral circulation that SP could elicit similar effects on the integrity of the blood-brain barrier.

Bradykinin

Bradykinin has been localised in nerve fibres and cell bodies of neurones in the hypothalamus and cerebral cortex [Wahl et al., 1986]. Bradykinin is vasoactive and has the ability to alter the permeability of blood vessels (in the periphery and the CNS) [Regoli & Barabe, 1980; Unterberg et al., 1984; Wahl et al., 1986]. The actions of bradykinin are mediated by one of two receptors- B_1 which are contractile or B_2 which are dilator [Regoli & Barabe, 1980; Regoli, 1984]. The receptors are localised on smooth muscle and endothelial cells [Edvinsson et al., 1993]. There are some species differences in the responses of cerebral blood vessels to bradykinin. In the canine, bradykinin causes a constriction of the major arteries while in man, feline, rabbit, elicits a vasodilator response [Edvinsson et al., 1993].

In the cat, bradykinin elicits a dose dependent dilatation following the microapplication or superfusion on cortical arterioles [Unterberg et al., 1983; Wahl et al., 1986]. In contrast, the cerebral veins demonstrate a constriction following prolonged superfusion [Unterberg et al, 1984]. Hypotheses explored to explain the above responses include suggestions that bradykinin may induce the swelling of the cerebrovascular endothelial cells [Unterberg et al, 1984]. The vasodilatation by bradykinin is prevented by superoxide dismutase and catalase indicating a role for oxygen radicals in the development of the responses [Kontos et al, 1984]. Intracerebroventricular administration of bradykinin increases CBF and glucose metabolism but has no effect on oxygen consumption [Unterberg et al., 1983].

Angiotensin

Angiotensin converting enzyme has been isolated in cerebral capillaries [Gimbrone et al., 1979]. Angiotensin binding sites have been identified in cortical and cerebellar microvessels [Changaris et al., 1978; Speth & Harik, 1985]. Angiotensin may be involved in the regulation of blood-brain barrier permeability to water. Angiotensin II is a constrictor of cerebral vessels in man, feline and rabbit (in the nanomolar range) [Edvinsson et al., 1979]. Microapplication or superfusion of

angiotensin II on cerebral arterioles reduces the calibre of cortical surface arterioles [Acar & Pickard, 1978; Wei et al., 1978]. However, vasodilatation induced by angiotensin II has been described in some species [Toda & Miyazaki et al., 1981]. The vasoconstrictor and vasodilatation induced by angiotensin II is endothelium mediated and are inhibited by cyclo-oxygenase inhibitors [Toda & Miyazaki, 1981]. Intracarotid infusion of angiotensin II reduces CBF and pial arteriolar calibre but these effects are not observed if the hypertensive response is attenuated suggesting an autoregulatory response [Edvinsson et al., 1993].

Concluding Remarks

The application of exogenous noradrenaline or neuropeptide Y on to cerebral vessels results in a vasoconstriction. The sympathetic system does not have any significant role in the regulation of basal cerebral blood flow. The physiologic significance of the sympathetic system in the cerebrovasculature is probably to facilitate the attenuation or prevention of excessive cerebral vasodilatation as may be experienced in conditions of systemic hypertension rather than a role in the physiologic control of cerebral blood flow.

The cholinergic innervation is present in the cerebrovascular bed and can modulate local cerebral blood flow directly (smooth muscle) or indirectly (endothelium). However, the role of the cholinergic system in cerebrovascular pathology is unclear at present and further investigations are necessary for evaluating its role.

There are a number of issues that arise from the description of peptides in the cerebrovasculature. It is known that most peptides would not be expected to cross the blood brain barrier, however, there is evidence that some of these peptides may regulate blood-brain barrier function. Additionally, the release of the peptides may be highly localised and thus play a role in regulating haemodynamics in the vicinity of their release. The release of vasodilator neuropeptides from the perivascular innervation may be significant in preventing excessive vasoconstriction of the cerebral vessels as may be experienced in subarachnoid haemorrhage. The trigeminal

innervation plays a role in the transmission of pain. The release of vasodilator peptides from this system may play a role in the aetiology of migraine. Certainly there is increasing interest in the developments of therapeutic agents that can antagonise or minimise the actions of the peptides since many of the peptides have been implicated to play a role in a variety of cerebrovascular disease including subarachnoid haemorrhage, ischaemia and migraine.

Endothelium Derived Factors

Endothelium derived relaxing factor (EDRF)

The importance of endothelium derived factors in the regulation of vascular tone has concentrated on endothelium derived relaxing factor (EDRF) [Furchgott & Zawadski, 1980; Moncada et al., 1991]. It has been suggested that EDRF may be nitric oxide (NO) or a nitric oxide containing moiety, e.g., Nitrosothiols [Faraci & Brian, 1994]. Nitric oxide is a freely diffusible and highly reactive mediator of vascular and non-vascular function and has a short half-life (~6 sec). Nitric oxide is synthesised from L-Arginine to form L- Citrulline and nitric oxide and the synthesis is catalysed by a family of enzymes called the nitric oxide synthases (NOS) [Faraci & Brian, 1994; Iadecola et al., 1994].

Three isoforms of NOS have been identified, 2 constitutive isoforms (neuronal NOS and endothelial NOS) and an inducible isoform (inducible NOS) [Nathan, 1992; Marletta, 1993]. Neuronal NOS (nNOS) and endothelial NOS (eNOS) are involved in the basal synthesis of nitric oxide. These enzymes are activated by elevations in intracellular calcium and facilitate the phasic production of nitric oxide [Moncada et al., 1991]. Neuronal NOS is primarily found in the cytosolic fraction while the endothelial NOS is a membrane associated enzyme [Nathan, 1992; Faraci & Brian, 1994]. Nitric oxide released from the endothelium produces vasodilatation by the activation of soluble guanylate cyclase within the vascular smooth muscle cells to increase cGMP [Moncada et al., 1991]. Soluble guanylate cyclase can be activated directly by the nitric oxide donors, e.g. sodium nitroprusside and glyceryl trinitrite to produce vasodilatation [Moncada et al., 1991; Archer, 1992].

The constitutive form of NOS has been localised in the cerebrovascular endothelium [Bredt et al., 1990; Nozaki et al., 1993]. The basal production of nitric oxide has an important role in the regulation of vascular tone in cerebral arteries and arterioles *in vivo* and *in vitro* [Marshall et al., 1988; Faraci & Brian, 1994]. A number of neurotransmitters (5-Hydroxytryptamine, Acetylcholine, Bradykinin, Endothelin, SP, ATP, Arginine Vasopressin, Histamine, Oxytocin) produce vasodilatation of cerebral arteries and arterioles [Faraci & Brian, 1994]. The inhibition of constitutive

nitric oxide synthase by L- Nitro Arginine (L-NOARG), L- Nitroarginine Methyl Ester (L-NAME) or L- Mono Methyl Nitroarginine (L-NMMA) produces increases in MABP and decreases in CBF [Faraci & Brian, 1994; Iadecola et al., 1994].

Nitric oxide is involved in the regulation of resting CBF and vasodilator responses in arteries and arterioles [Faraci & Brian, 1994]. The removal of the vascular endothelium results in a loss of vasodilator responses. NOS containing neuronal fibres have been identified in the sphenopalatine ganglion and activation of these nerves elicits a vasodilator response [Iadecola et al., 1994]. Nitric oxidergic perivascular nerves innervating cerebral arteries and arterioles have been described.

NO independent vasodilatation of cerebral arteries and arterioles can be myogenic in origin and is observed during cerebral autoregulation [Iadecola et al., 1994]. The role of NO in preventing platelet aggregation or leucocyte activation in the vascular endothelium has been well documented [Nishimura et al., 1991; Rosenblum et al., 1992]. This property of nitric oxide is important for the maintenance of microvascular flow in the brain.

The enzyme has been localised to dendrites and axons of nerves associated with cerebral microvessels [Faraci & Brian, 1994]. Stimulation of nuclei such as the cerebellar fastigial nucleus has demonstrated NO mediated increases in CBF [Iadecola, 1992; Iadecola et al., 1994]. The activation of NMDA and non-NMDA receptors may stimulate NO production and its release into the extracellular space [Faraci & Breese, 1993; Faraci & Brian, 1994]. The presence of NOS has also been demonstrated in perivascular nerves innervating cerebral arteries and arterioles.

Constitutive NOS activation by receptor mediated mechanisms have been observed in astrocytes [Aoki et al., 1991; Murphy et al., 1993]. Calcium ionophore A23187, bradykinin, glutamate (via NMDA and non-NMDA receptors) and noradrenaline have all triggered the synthesis of NO [Faraci & Brian, 1994]. Induction of iNOS in astrocytes and microglia by lipopolysaccharide and cytokines indicates a role for NO in the progression of neuronal damage following cerebrovascular injury [Faraci & Brian, 1994].

Prostacyclin & Thromboxane A_2

Prostacyclin (PGI_2) is produced from the conversion of prostaglandin H_2 by prostacyclin synthetase. Prostacyclin is a vasodilator that acts on peripheral and cerebral arteries and arterioles. Low concentrations of prostacyclin dilate arteries and arterioles while high concentrations (μM) can constrict some blood vessels [Toda, 1980]. Prostacyclin can reverse the vasoconstrictor effects of 5-HT, Noradrenaline, Angiotensin II, $PGF_{2\alpha}$ and Thromboxane A_2 [Paul et al., 1982]. The mechanism of action in cerebral arteries is unclear but in peripheral arteries prostacyclin stimulates the production of cAMP. The relaxant effects of prostacyclin are not abolished by the removal of the vascular endothelium (unlike nitric oxide) [Toda et al., 1988]. There is evidence that the synthesis of prostacyclin may not be endothelium dependent and PGH_2 can be converted to PGI_2 in the vascular smooth muscle.

Thromboxane A_2 is a potent constrictor of cerebral arteries. Thromboxane A_2 is produced from the metabolism of arachidonic acid by PLA_2. The thromboxane A_2 mediated constrictions are mediated via calcium channels (constrictions inhibited by the calcium channel antagonists verapamil, nifedipine and nimodipine). Thromboxane A_2 can increase intracellular calcium by increased influx through receptor operated calcium channels and the release calcium from intracellular stores [Wendling & Harakal, 1991].

1.2 Cerebrovascular disease

1.2.1 Cerebrovascular disease in man

Stroke is the third leading cause of mortality, following heart disease and cancer, in the western world. Stroke, as defined by the World Health Organisation, is :

"rapidly developed clinical signs of focal disturbance of cerebral function of presumed vascular origin and of more than 24 hours duration".

This definition incudes most forms of intracerebral haemorrhage, subarachnoid haemorrhage and cerebral infarction (with or without demonstrable arterial occlusion) but does not include transient ischaemic attacks (TIA's) which by definition last less than 24 hours.

There are two general types of strokes, infarction (70%) and haemorrhagic (30%), and can be classified into further subtypes [Sacco, 1993]. Approximately 44% of infarcted strokes are thrombo-embolic in origin and two thirds of these involve the occlusion of the large cerebral (ACA, MCA) arteries leading to cerebral infarction [Sacco, 1993]. Lacunar infarcts which result from the obstruction of the small penetrating arteries and arterioles and the infarction of the associated deep brain areas. There are multiple sources for thrombo-emboli and include cardiac disease, carotid artery or vertebro-basilar artery disease. Haemorrhagic strokes result from intracerebral or subarachnoid haemorrhage. The aetiology of these haemorrhagic stroke include chronic hypertension and ruptured aneurysms.

Subtypes of ischaemic stroke

Thrombotic stroke

The aetiology of this form of stroke is thought to be the presence of artherosclerotic lesions found at the bifurcations and curves of the larger extracranial vessels [Mohr & Sacco, 1992]. The location of the artherosclerotic plaque is thought to be at proximal sites within the vessel (e.g. division of the common carotid artery

into the internal carotid arteries and external carotid arteries). The increase in the size of the artherosclerotic lesions lead to a progressive stenosis of the arterial lumen and eventually occlusion occurs due the formation of a thrombus within the vascular lumen.

The mechanism for the development of stroke following this lesion is thought to be the inadequacy of perfusion at sites distal to primary occlusion or stenosis [Bogousslavsky & Regli, 1986]. The main reason for this is the response of the collateral vessels to undergo dilatation to compensate for the reductions in CBF. If the resistance arterioles are maximally dilated and there is complete occlusion of the carotid artery, the arterial territory is at risk of cerebral ischaemia and eventually leading to infarction [Mohr & Sacco, 1992]. The main brain regions at risk from the carotid artery stenosis are the suprasylvian, frontal, central and parietal portions of the cerebral hemisphere [Torvick, 1984; Mohr & Sacco, 1992]. Stenosis in the vertebro-basilar system puts the occipital pole at risk.

Embolic Stroke

Embolic stroke constitutes approximately 15-30% of all stroke cases [Sacco, 1993]. The common origin of this type of stroke is the formation of emboli from the site of the carotid artery stenosis [Mohr & Sacco, 1992]. The embolism affects the territory of the occluded artery (commonly middle cerebral artery) [Mohr & Sacco, 1992]. The sites of thrombi formation in the heart include the heart valves, atrial fibrillation or ventricular fibrillation [Mohr & Sacco, 1992; Koudstaal, 1993]. The emboli are formed of fragments of thrombi or platelet aggregates. The friability of the thrombi and platelets, the size of embolus and the decreasing size of the arteriolar lumen and bifurcation of the vascular tree will determine the site of arrest [Mohr & Sacco, 1992; Sacco, 1993]. The common sites for the lodging of the emboli are in arterioles, however, larger emboli composed of fibrin - platelet complexes may obstruct the larger cerebral arteries such as the middle cerebral artery.

The morphology of the occluded vessels indicate that although vascular distension is observed there is minimal damage to the vascular wall. The vascular

endothelium in these cases does not seem to be injured and may not be the primary mediator of the pathogenesis of embolic stroke.

Lacunar strokes

Lacunar infarcts account for approximately 20% of stroke cases [Sacco, 1993]. A lacunar infarct is defined by a "small, deep infarct attributable to a primary arterial disease that involves a penetrating branch of a large cerebral artery" [Mohr & Sacco, 1992]. The size of lacunar infarcts is usually small and is largely dependent on the distribution territory of the occluded vessel [Mohr & Sacco, 1992]. The vessels that are normally affected are 100-400μm in diameter and are located in the deep brain regions that are supplied by the penetrating arterioles e.g. Basal ganglia (caudate , putamen), thalamus, internal capsule, pons and white matter tracts of cerebral gyri [Mohr & Sacco, 1992, Bogousslavsky, 1993]. Lacunar infarcts have rarely been observed in cortical gray matter, corpus callosum, medulla or spinal cord [Mohr & Sacco, 1992]. Most lacunar infarcts are observed in the territory of the lenticulostriate branches of the MCA, and the ACA and paramedian branches of the basilar artery (supplying the pons) [Bogousslavsky, 1993]. The absence of collateral vessels in the deep areas of the brain, unlike the cerebral cortex, make these areas more susceptible to ischaemic damage. Occlusion of the penetrating arterioles may be caused by microartheromas, lipohyalinosis, microemboli or the result of haemodynamic disorders such as polycythaemia and arteritis [Mohr & Sacco, 1992]. The risk factors for the development of lacunar infarcts are broadly similar for the development of stroke in general (see section on Risk Factors).

Haemorrhagic strokes

Intracerebral haemorrhage

An intracerebral haemorrhage occurs as a result of bleeding from arteries or arterioles directly into the brain parenchyma. Intracerebral haemorrhage contributes 5-15% of all strokes and the incidence increases with age [Sacco, 1993; Boysen, 1993].

Spontaneous intracerebral haemorrhage occurs predominantly in the deep portions of the cerebral hemispheres (putamen, subcortical white matter, thalamus, cerebellum and pons) [Kase et al., 1992]. The intracerebral haemorrhage is usually the result of bleeding from the arterioles of 100-400μm diameter (similar to the vessels affected in lacunar infarcts) and occur predominantly in the vascular distribution territories of the small perforating cerebral arterioles (lenticulostriate, thalamo, basilar artery branches) [Kase et al., 1992]. The rupture of these vessels in conditions such as chronic hypertension can result in the development of intracerebral haemorrhage.

Mechanisms of vascular rupture are unclear but alterations in the arteriolar morphology (lipohyalinosis) have been implicated in the pathogenesis of intracerebral haemorrhages, e.g. lesions of the intimal layer in cerebral vessels can lead to bleeding into medial /adventitial layer thus weakening the vascular wall [Kase et al., 1992]. Secondary bleeding as result of damage to the smaller vessels could be responsible for enlarging the size of the haematoma [Kase et al., 1992]. Rupture of microaneurysms or vascular weakness as a result artherosclerosis are mechanisms that may be involved in the production of an intracerebral haemorrhage.

General consensus is that the bleeding associated with an intracerebral haemorrhage is a monophasic event, however, the use of anticoagulants or vascular malformations may facilitate an increase in the volume of the haematoma [Boysen et al., 1993; Kase et al., 1992]. The increase in bleeding and size of the haematoma can lead to the herniation of the brain across the midline or the distribution of blood through the ventricular system thus increasing the pressure on the brain stem.

Although hypertension has been cited as the major risk factor for the development of intracranial haematoma, intracerebral haemorrhage in patients in the absence of hypertension have been reported [Boysen, 1993]. Vascular malformations (angiomas) that maybe arterio-venous, venous or cavernous, may rupture and lead to bleeding in the brain parenchyma [Kase et al., 1992; Boysen, 1993]. The use of sympathomimetic drugs, anticoagulants agents, fibrinolytic agents may be some of the other conditions that may result in the development of an intracerebral haemorrhage. Risk factors for the development of intracerebral haemorrhage include hypertension,

which is the leading risk factor, blood dyscracies, vascular malformations and tumours.

Subarachnoid haemorrhage

Subarachnoid haemorrhage contributes approximately 13% of total stroke cases [Brown & Wiebers, 1993]. The common causes for the development of subarachnoid haemorrhage are traumatic (head injury), aneurysm rupture, vascular malformations and bleeding disorders [Brown & Wiebers, 1993]. Other causes include vasculitis, drug abuse, cerebral bacterial infections, cerebral sinus thrombosis and secondary bleeding as result of an intracerebral haemorrhage [Mohr et al., 1992]. The blood leaks into the subarachnoid space and is in direct contact with CSF. The diagnosis of subarachnoid haemorrhage normally displays evidence of clotted blood over the ventral surface of the brain [Brown & Wiebers, 1993; Mohr et al., 1992]. The arachnoid membrane is very resistant to the pressure of CSF and hence a large amount of blood is required to rupture the subarachnoid membrane, hence, the blood is confined in the subarachnoid space.

Cerebral vasospasm is a major complication and leading cause of cerebral ischaemia or infarction following a subarachnoid haemorrhage [Brown & Wiebers, 1993; Mohr et al., 1992]. The aetiology for the development of cerebral vasospasm is unclear but the vessels affected display reduced calibre and thrombosis, although, these may be symptomatic and not causal of subarachnoid haemorrhage. Cerebral vasospasm following subarachnoid haemorrhage is typically observed 3-7 days following the initial insult, but, some investigators have reported vasospasm 7-14 weeks following the event [Brown & Wiebers, 1993; Mohr et al., 1992]. The vessels normally affected are those comprising the circle of Willis (basilar artery may also be affected) and the vasospasm spreads along the vessel.

Risk factors for stroke

The strongest determinant of stroke is age with the incidence of stroke increasing exponentially with age [Bamford et al., 1990; Sacco, 1993]. The majority

of strokes occur in patients over the age of 65. Other risk factors for increasing stroke incidence include gender, ethnic background and heredity. The incidence of stroke is between 16% and 70% higher in men than in women [Sacco et al., 1991; Sacco, 1993]. Evidence from epidemiological studies in United States, Taiwan and U.K. have indicated that stroke incidence may be related to ethnicity [Sacco, 1993]. The genetic contribution to the risk of stroke is an area of some controversy since the link between modifiable environmental factors and genetics factors has not been isolated.

There are a number of modifiable risk factors for stroke including hypertension, diabetes, cardiac disease, hypercholesterolaemia, smoking and alcohol use [Shaper et al., 1991; Wolf et al., 1992; Higa & Davanipour, 1991]. The association of these risk factors varies with the subtypes of stroke examined. Hypertension is the second most common risk factor for the development of stroke[Shaper et al., 1991]. The risk of stroke increases proportionately with increases in systemic blood pressure [Shaper et al., 1991, Wolf et al., 1992]. Systolic blood pressure between 160-180 mmHg produced a four fold increase in stroke risk while systolic blood pressures above 180 increases the risk six fold. Hypertension has a strong association with cerebral infarction, intracerebral haemorrhage and subarachnoid haemorrhage.

Cardiac diseases, such as atrial fibrillation, valvular heart disease, myocardial infarction, coronary artery disease, congestive heart failure and ventricular hypertrophy, have been associated with an increase in the risk of stroke [Wolf et al., 1991]. Atrial fibrillation increases the stroke risk five fold. Coronary artery disease doubles the stroke risk while cardiac failure increases the risk four fold. Cardiac disease is primarily associated with cerebral infarction and not associated with haemorrhagic stroke [Sacco, 1993].

Diabetes mellitus increases the risk of stroke , although, if hypertension cardiac disease and hypercholesterolaemia were treated then the stroke risk due to diabetes was reduced [Shaper et al., 1991]. Elevations in serum lipids (triglycerides, cholesterol, LDL, HDL) are recognised risk factors for artherosclerosis and cardiac disease [Qizilbash et al., 1992]. The role of serum lipids in the development of stroke is unclear. The formation of artherosclerotic plaques is also associated with carotid

artery diseases. Carotid artery disease can lead to the stenosis of the vessel, although, non-stenosing plaques do occur. The adequacy of the collateral circulation, the degree of carotid artery stenosis and the propensity to form thrombi at the site of stenosis can affect the incidence of stroke.

Cigarette smoking has been established as a risk factor for cardiac diseases [Higa & Davanipour, 1991]. More recent investigations have suggested that cigarette smoking may be a risk factor for stroke [Higa & Davanipour, 1991]. The incidence of stroke increases in heavy smokers and with an even higher risk when combined with hypertension. This risk is greater than the incidence rate for cerebral infarction. The association of alcohol and stroke risk is controversial. The investigations carried out have demonstrated strong associations and others have demonstrated the absence of any link [Sacco, 1993]. The association in haemorrhagic stroke (intracerebral haemorrhage and subarachnoid haemorrhage) is more established. Other risk factors for the development of stroke under investigations include the use of migraine and oral contraceptive formulations. Haematological disorders such as sickle cell anaemia, altered haematocrit, polycythaemia and fibrinogen levels may be potential risk factors for the development of stroke [Sacco, 1993].

1.2.2 Pathophysiology of cerebral ischaemia

Alterations in ion fluxes and energy metabolism

In cerebral ischaemia, cerebral autoregulation is impaired and as a result CBF varies passively with increasing MABP [Symon et al., 1976]. In humans and larger animals, spontaneous electrical activity ceases at a flow threshold of approximately <20 ml/100g/min [Heiss , 1992; Branston et al., 1974]. At a flow threshold of <10-12 ml/100g/min there is a loss of ion homeostasis within neurones and glial cells [Astrup et al., 1977; Branston et al., 1977] (Figure 2). The ionic changes that ensue at these levels of flow are preceded by an increase in the extracellular K+ concentration. Under anoxic conditions there is an efflux of K^+ and an intake of Ca^{2+}, the reasons for these changes include the collapse in membrane function especially the cessation of ionic

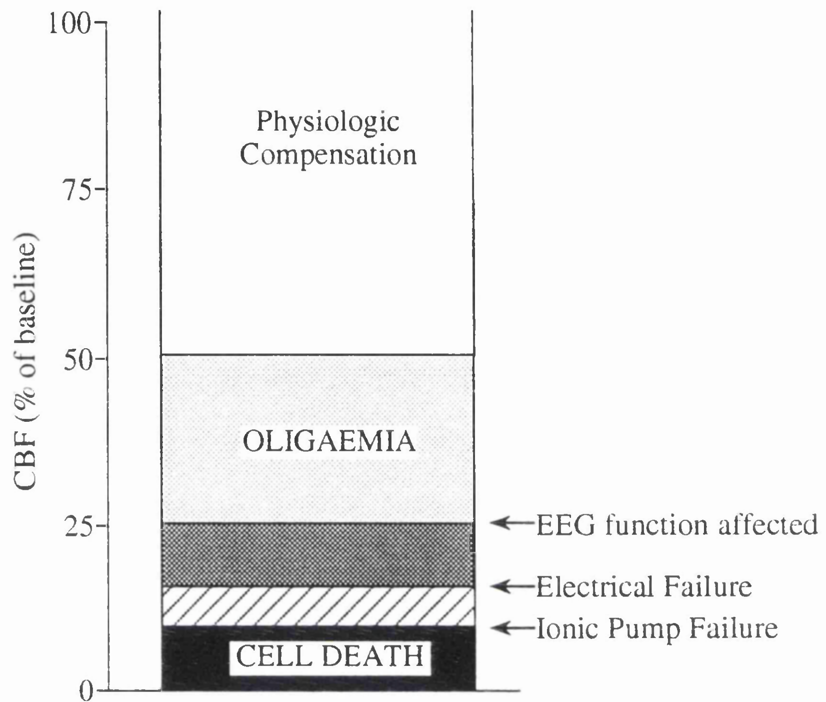


Figure 2 Schematic diagram illustrating the thresholds for reductions in CBF following cerebral ischaemia resulting in neuronal dysfunction and death (Figure adapted from Astrup etal., 1977)

pump activity [Harris et al., 1981; Nicholson et al., 1977]. The mechanism of cessation of membrane function is discussed later.

The gross alterations in neuronal functions is the result of the loss of the physiologic gradients of the ions [Naritomi et al., 1988]. An important distinction between thresholds for functional impairment and the development of infarction is that cerebral infarction varies and increases with the duration of ischaemia [Jones et al., 1981; Heiss et al., 1983] (Figure 3). In transient cerebral ischaemia, a flow level of <10-12 ml/100g/min for 1-3 hours leads to cerebral infarction [Jones et al., 1981]. Under conditions of permanent cerebral ischaemia, a level of <17-20 ml/100g/min is required for the development of cerebral infarction [Jones et al., 1981]. In small animals such as rodents the flow threshold for ischaemic neuronal damage occurs at higher levels. The probable reasons for the higher levels are increased neuronal packing density and higher cerebral metabolic rate which leads to a higher basal CBF [Kaplan et al., 1991; Naritomi et al., 1988; Tyson et al., 1984]. The flow thresholds for the impairment of cell function would occur at a similar percentage reduction in CBF in the primate and rodent.

Although perturbations in cellular metabolism occur at the very low levels in CBF, alterations in other cerebral function occur at higher flow thresholds. Intracellular and extracellular acidosis as result of lactate accumulation occurs at higher flow thresholds and pO_2 levels [Siesjo, 1992a]. Cerebral oedema can develop at high levels of CBF primarily as a result of increased MABP [Ito et al., 1989; Siesjo, 1992a]. Protein synthesis is the cerebral function most sensitive to alterations in CBF [Mies et al., 1991; Heiss, 1992].

Energy metabolism and ion homeostasis are an integral part of the functions of neurones. The maintenance of ionic gradients across neural membranes is primarily carried out by active transport. ATP produced during aerobic respiration is used to drive Na^+/K^+ exchanger and Ca^{2+}/H^+ exchanger [Siesjo, 1992a]. The active transport of these ions leads to a 10 fold higher level of Na+ and a 10000 fold higher Ca^{2+} level in the extracellular space and a 40 fold higher K^+ concentration in the intracellular space. During neuronal depolarisation, the increase in intracellular Na+ occurs as

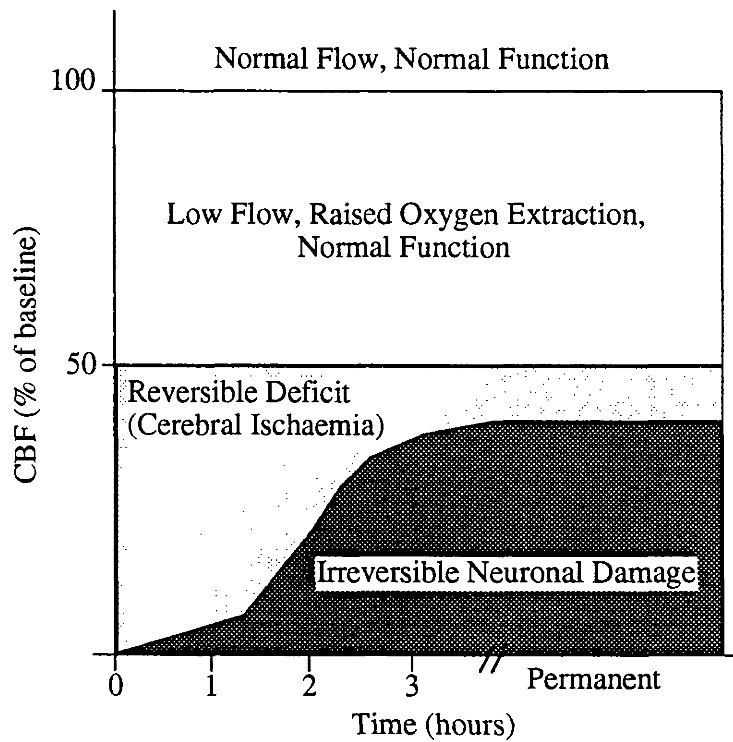


Figure 3 Schematic diagram illustrating the relationship between the severity of reduction in CBF and duration of cerebral ischaemia and the development of irreversible neuronal damage(Figure from Jones et al., 1981)

result of entry through conductance channels or in exchange for Ca^{2+} /H^+. The calcium gradient is maintained by a Na^+/Ca^{2+} ion exchanger which facilitates the extrusion of calcium while the extrusion of H^+ is carried out by an Na^+/H^+ pump [Siesjo, 1992a]. The membrane potential is the main determinant of Na^+/Ca^{2+} exchange and under normoxic conditions the active and passive ion fluxes are maintained [Siesjo, 1992b].

Under conditions of ischaemia where there is ionic pump failure, membrane depolarisation is followed by the flow of ions down their concentrations gradients, i.e., influx of Na^+ and Ca^{2+} into the neurones and an efflux of K^+ and Cl^- [Hansen, 1985]. In the initial phase of ischaemia, there is a slow efflux of K^+ from neurones and a reduction in extracellular pH (the reduction in pH is probably due to the formation of lactic acid) [Siesjo et al., 1992b]. The second phase of ionic changes sees the efflux of K^+ and influx Na^+/Ca^{2+} and osmotically obligated water leading to swelling of the neurones [Hansen, 1985].

The synthesis of ATP is downregulated during ischaemia/hypoxia but the utilisation of ATP is unaffected [Siesjo, 1988]. The hydrolysis of ATP results in elevations of ADP levels. The ADP produced can be used in conversion of phosphocreatine to creatine and the production of ATP. This pathway of ATP production is maintained until phosphocreatine levels are depleted. The transition from aerobic to anaerobic respiration sees the production of ATP and lactate but the ensuing energy failure cannot be prevented. The reductions in ATP levels in ischaemia are the attempts of neurones to maintain their ionic gradients.

A number of authors have described alterations in brain pH associated with cerebral ischaemia [Siesjo, 1992a]. In general, there is a drop of approximately 0.6-1 pH unit during cerebral ischaemia [Behar et al., 1989]. In transient cerebral ischaemia with reperfusion, the magnitude of pH reduction is decreased during the reperfusion phase. The reductions in pH may be due to increased lactate production or a decrease in the CMR_{glc}. At pH levels below 6.1 there is an arrest of oxidative phosphorylation [Paschen et al., 1987].

In focal cerebral ischaemia there is a greater decrease in pH in the core of the lesion than in the ischaemic penumbra [Paschen et al., 1987]. Reductions in extracellular/intracellular pH is due to a mismatch between glycolysis and oxidative phosphorylation [Siesjo, 1992a]. During hypoxia/ischaemia, the mitochondria lose their ability to sequester Ca^{2+} ions (energy dependent process) and this results in an increase in intracellular calcium levels [Siesjo & Bengtsson, 1989]. Hyperglycaemia exacerbates acidosis due to increased substrate availability but respiration is anaerobic [Chopp et al., 1988].

During complete or near complete ischaemia (global ischaemia) there is a cessation in the delivery of substrate (glucose) to the brain and rapidly leads to energy failure [von Hanwehr et al., 1986; Chopp et al., 1987]. Pre-ischaemic hyperglycaemia exacerbates ischaemic damage in global or forebrain ischaemia [Chopp et al., 1988]. The neuronal damage is associated with oedema, post-ischaemic seizures and rapidly maturing brain lesions [Siesjo et al., 1992b]. In transient focal ischaemia, hyperglycaemia increases infarct size [Siesjo et al., 1992b]. In permanent ischaemia the effects of hyperglycaemia are more ambiguous, some authors have reported an increase in the lesion size while others have reported either no change or a reduction in lesion size [Ginsberg et al., 1987; Nedergaard & Diemer, 1987; de Courten-Myers et al., 1989]. The mechanism by which hyperglycaemia exerts its effects in ischaemia are unclear but the destruction of cerebral microvessels due to acidosis has been suggested (absence of proliferating vessels in the border between infarct and non-infarcted tissue) [Nedergaard & Diemer, 1987].

Mechanisms of Ischaemic Neuronal damage

Calcium

Increases in intracellular calcium has implicated this ion as an initiator of the ischaemic cascade eventually leading to neuronal death [Siesjo, 1988; Siesjo, 1992a; Siesjo, 1992b]. Under resting conditions there is a 10000 fold excess of calcium in the extracellular space and the concentration gradient is maintained by the means of

electrogenic pumps (Ca^{2+}/Na^+ exchanger). Intracellular calcium is sequestered by the endoplasmic reticulum and mitochondria.

Increases in intracellular calcium can arise from a number of sources, increased influx of calcium or reduced efflux is one aspect of calcium homeostasis [Sharma & Hakim, 1993]. Increased release of calcium from intracellular stores as a result of reduced sequestration or reduced binding also contribute to the increase in intracellular calcium [Siesjo & Bengtsson, 1989]. The depolarisation of neurones leads to the influx of calcium and increases in intracellular calcium lead to the activation of transcription factors and induce the expression of immediate early genes or stress proteins [Hisanaga & Hirokawa, 1990; Siesjo, 1992b]. The marked increases in intracellular calcium during cerebral ischaemia may be detrimental due to increased energy failure and the inability of the electrogenic pumps to maintain the ionic gradient [Siesjo & Bengtsson, 1989; Stys et al., 1991].

Calcium entry from the extracellular space can occur vià voltage sensitive calcium channels or receptor operated calcium channels [Siesjo & Bengtsson, 1989]. The release of calcium from intracellular stores occurs primarily via G-protein coupled receptors which stimulate phospholipase C and the production of diacylglycerol (DAG) and inositol triphosphate (IP_3) [Siesjo, 1992b]. IP_3 initiates the release of calcium from the endoplasmic reticulum [Siesjo & Bengtsson, 1989]. Glutamate acting through the metabotropic receptor may initiate the increase in intracellular calcium [Nicoletti et al., 1986; Sladeczek et al., 1988]. Intracellular calcium is inactivated by its binding to specific proteins such as calmodulin and calcibindin or sequestration into the endoplasmic reticulum or mitochondria [McBurney & Neering, 1987].

An increase in intracellular Ca^{2+}, through voltage sensitive channels or receptor operated channels, following hypoxia/ischaemia is thought to trigger neuronal necrosis [Choi, 1988]. Increases in intracellular calcium lead to the activation of lipases, proteases, endonucleases and protein kinases and results in the alterations in membrane function [Siesjo et al., 1992b]. Calcium mediated protease activation can result in the breakdown of the cytoskeleton. An important aspect on increased

intracellular calcium levels is the activation of phospholipases [Siesjo et al., 1992b]. Phospholipid hydrolysis by phospholipase A_2 results in the formation of free fatty acids, lysolipids and arachidonic acid [Siesjo et al., 1992b]. The lysophospholipid and free fatty acids may cause membrane damage. The activation of phospholipase C results in the formation of IP_3 and DAG and DAG undergoes metabolism to form arachidonic acid [Siesjo et al., 1992b]. Metabolism of arachidonic acid by cyclo-oxygenase and lipoxygenase results in the formation of metabolites that can cause vasomotor disturbances, blood-brain barrier breakdown, free radical release and the activation of protein kinases.

Excitatory Amino Acids

The excitatory amino acid glutamate is one of the major neurotransmitters in the brain. Ischaemic neuronal damage is characterised by the damage to neuronal cell bodies but the sparing of axons [Olney, 1978; Choi, 1988]. Axonal sparing allows the release of neurotransmitters from the terminals and thus exacerbating the neuronal damage. The physiologic role of these transmitters and receptors is varied. Amongst the possible roles are long term poteniation (LTP), burst firing of CNS neurones and some aspects of learning and memory [Collingridge & Bliss, 1987; Herrling et al., 1983]. The main amino acids are glutamate and aspartate and the actions of these substances are mediated via excitatory amino acid receptors. There are five types of receptors classified on the basis of selective agonists and these are the NMDA receptors, AMPA receptors, kainate receptors, metabotropic receptors and the L-AP4 receptor [Choi, 1990]. The NMDA, AMPA and Kainate receptors are linked to ion channels- Na^+, K^+ and Ca^{2+} permeable [Siesjo, 1981; Gill, 1994]. The metabotropic receptor is linked via a G-protein to phospholipase C while the transduction mechanism for the L-AP4 receptor is suggested to be via cGMP [Choi, 1990]. The excitotoxic hypothesis has implicated glutamate as a mediator of neuronal damage following cerebral ischaemia [Benveniste, 1991].

The NMDA receptor is the most widely studied of the excitatory amino acid receptors. Under normal physiologic conditions, the NMDA receptor ionophore is

blocked by Mg^{2+} ions [Foster & Fagg, 1987]. This blockade is voltage dependent and the depolarisation of the cell membrane relieves the Mg^{2+} blockade and allows the influx of calcium and sodium ions [Foster & Fagg, 1987; Choi, 1988]. The NMDA receptor complex comprises a number of distinct sites that allow the modulation of receptor activity [Foster & Fagg, 1987]. Activation of the NMDA receptor can be attenuated by the competitive inhibition of the neurotransmitter recognition site by antagonists (CGS19755, D-CPP-ene) [McCulloch et al., 1991]. Non-competitive antagonism of sites within the ionophore is usually use dependent by agents (MK801, PCP) and decrease the activity of this receptor [McCulloch et al., 1991]. Allosteric modulatory sites also exist on the receptor complex and these are the strychnine insensitive glycine site (positive modulation site), which is antagonised by L-687414, ACEA-1021 and a polyamine site antagonised by ifenprodil and eliprodil. Antagonism of the allosteric sites reduces the activity of the NMDA receptor [Muir & Lees, 1995].

The non-NMDA receptors (AMPA and Kainate) are mediators of fast synaptic transmission and activation of these receptors facilitates membrane depolarisation and alleviation of the Mg^{2+} block in the NMDA channel. The ion channels associated with these receptors are permeable to Na^{+} and K^{+} although there is some evidence that calcium may also enter through the ion channels [Choi, 1988; Hollman et al., 1991].

The role of glutamate in the genesis of ischaemic cell injury has been demonstrated *in vitro* and *in vivo* [Choi, 1990; Benveniste, 1991]. Lucas and Newhouse (1957) demonstrated the neurotoxicity of excitatory amino acids to retinal neurones. Subsequent studies demonstrated that increasing concentrations of glutamate were neurotoxic to neuronal cell cultures and the cell necrosis was associated with a lethal influx of Ca^{2+} as a result of overactivation of glutamate receptors [Choi, 1990; Benveniste, 1991]. Increases in the extracellular concentrations of glutamate have been observed in global and focal cerebral ischaemia and the extent of glutamate increases have been correlated with the degree of neuronal damage [Benveniste et al., 1984]. Further evidence was provided by the neuroprotective efficacy of NMDA and non-NMDA receptor antagonists in experimental models of ischaemia [McCulloch et al., 1991; Gill, 1994; Muir & Lees, 1995].

Free Radicals

Apart from glutamate, other mediators of ischaemic neuronal damage include the free radicals [Phillis, 1994]. The source of free radicals has been speculated upon but arachidonic acid metabolism is thought to be a major source of free radicals [Siesjo, 1992b, Phillis, 1994]. Other sources include hyperoxia, impaired metabolism within the mitochondria and unchelated iron [Phillis, 1994]. The release of free radicals activates lipid peroxidation and can lead to membrane damage [Phillis, 1994]. Most cells possess endogenous free radical scavengers such as vitamins (A and C), glutathione peroxidase and superoxide dismutase (SOD). In experimental forebrain ischaemia there is increased lipid peroxidation and SOD production and the production of free radicals is dependent on oxygen tension [Siesjo, 1992b; Phillis, 1994]. During reactive hyperaemia following transient ischaemia, there is an increase in the production of free radicals due to an increase in the oxygenation of the ischaemic territory.

The Ischaemic Penumbra

The lesion following focal cerebral ischaemia comprises a central core region with dense ischaemia and a peri-focal region with less dense ischaemia, also termed the ischaemic penumbra [Astrup et al., 1981; Hakim et al., 1992]. The central core of the lesion normally comprises the caudate nucleus and some neocortical areas that are normally supplied by perforating arteries from the MCA and its lenticulostriate branches while the peri-focal areas are supplied by collateral anastomoses between the arterioles of the anterior cerebral artery (ACA), posterior cerebral artery (PCA) and the MCA [Strong et al., 1983a & 1983b].

The differences in the flow thresholds for reductions in spontaneous electrical activity and dysfunctions in ion homeostasis has led to the description of the ischaemic penumbra as an area where neurones are inexcitable but viable [Astrup et al., 1981; Strong et al., 1983a & 1983b]. The development of the ischaemic lesion is dependent on duration of the ischaemic period and the level of CBF maintained during

the ischaemic period [Jones et al., 1981]. The peri-focal areas may be viable for longer periods of time provided adequate blood flow is maintained from the collateral blood vessels [Heiss, 1992; Siesjo, 1992a]. Failing this the peri-focal areas are recruited into the ischaemic territory. Peri-focal areas can be salvaged by reperfusion or pharmacologic interventions that either increase CBF or prevent neuronal death [McCulloch et al., 1991; Gill, 1994].

Delineation of the penumbral areas is difficult. The peri-focal areas have blood flow, decreased electrical activity, efflux of K^+ and scattered neuronal necrosis, i.e., at early time points it is difficult to differentiate penumbral tissue from the ischaemic core [Strong et al., 1983a & 1983b; Heiss, 1992]. During the therapeutic window of opportunity there is a mismatch between the CBF and metabolic demands of the neurones [Heiss, 1992]. This leads to the release of inhibitory substances that can act initially to quell the metabolic demand e.g. GABA, Adenosine [Shimada et al., 1990; Matsumoto et al., 1992]. A sustained period of ischaemia can eventually overcome this mechanism and the release of glutamate and free radical production leads to the damage of neurones in the core and recruitment of peri-focal (penumbral tissue) into the ischaemic infarct [Siesjo, 1992a].

The patterns of ischaemic neuronal damage in transient global or forebrain ischaemia are different because the ischaemic period is transient and associated with a marked reduction in CBF [Pulsinelli et al., 1982b; Smith et al., 1984]. The marked reduction in CBF in global ischaemia results in the loss of membrane function and ionic homeostasis [Siesjo, 1992b].

Cerebral Blood Flow and Cerebral Ischaemic Damage

The impairment of neuronal function is dependent on the level of cerebral blood flow and the duration of the reduction in cerebral blood flow. In the ischaemic territory there is a failure of the autoregulatory capacity of cerebral blood vessels. Thus, increasing cerebral blood flow in the ischaemic territory provides a mechanism for reducing ischaemic brain damage. The use of vasodilator agents, such as L-type calcium channel antagonists and papaverine could have the effect of increasing

cerebral blood flow in the ischaemic territory above the thresholds for the development of ischaemic damage [Date & Hossmann, 1984; Kazda et al., 1982; Hossmann et al., 1973; Brandt et al., 1983] (Figure 2). However, these agents induce systemic hypotension and cerebral blood flow could be reduced and ischaemic brain damage could be exacerbated.

The regulation of cerebral blood flow is critical in the post-ischaemic phase. The administration of vasodilator agents may facilitate the increase of cerebral blood flow into the ischaemic territory. However, the reductions in cerebral blood flow are the result of complex mechanisms. There has been speculation on the mechanisms resulting in sustained reduction in cerebral blood flow including, active vasoconstriction, increased tissue oedema causing the obstruction of collateral vessels and occlusion of the microvasculature have been suggested mechanisms [Hatashita & Hoff, 1986; Dirnagl et al., 1994; del Zoppo, 1994]. The use of vasodilator agents such as nimodipine assumes that the alleviation of the vasoconstriction will facilitate the increasing cerebral blood flow above the thresholds for the development of neuronal damage. However, recent evidence has indicated that the occlusion of the cerebral microvasculature by the platelets and leucocytes may be a significant mechanism for the reductions in cerebral blood flow. The activation of cell adhesion molecules may serve to exacerbate the occlusions of the microvasculature and the role of the intercellular adhesion molecules in cerebral ischaemia is being explored.

1.2.3 Experimental models of cerebral ischaemia

Experimental models of cerebral ischaemia are an important aspect for the understanding of the ischaemic process. Although the models of ischaemia used are wide and varied, an important factor linking them is their reproducibility. Models of ischaemia have been developed to examine cerebrovascular and neuronal alterations following permanent focal ischaemia, transient focal ischaemia, transient global cerebral ischaemia and models of head injury such as subdural haematomas.

A number of important issues are raised when deciphering the descriptions such as the species used, the type of ischaemic injury and the physiologic status of the

animals. As described in an earlier section, there are a number of inter-species differences in the cerebrovascular anatomy. The structure of the circle of Willis as seen in the gerbil compared to other species, the anastomotic links between the ACA, MCA and PCA and the resulting collateral channels have implications for the severity and extent of ischaemic damage [Coyle & Jokelainen, 1982; Mchedlishvili & Kuridze, 1984]. The type of ischaemic insult, the duration of ischaemia and the size of the ischaemic penumbra are important for the neuroprotective strategies [Jones et al., 1981]. Since these factors determine the level of CBF which are important for the various neuronal functions (see previous section).

The factors mentioned above become less significant because the majority of studies are carried out in a small number of species with the rat being the most common. One of the reasons for the use of this species is the ease of the development of the model and the cost of conducting the experiments. Despite the apparent standardisation of the models of ischaemia, there is a great deal of variability between the various groups carrying out the studies. The physiologic status of the animal (arterial blood gas status, plasma glucose levels, haematocrit, body and brain temperature and MABP) are important contributors to the variability.

Reductions in the arterial pO_2 levels outwith the autoregulatory limits (<50 mmHg) results in an increase in CBF and at levels below 20 mmHg result in the cessation of cerebral function [Paulson et al., 1990]. In contrast an increase in pCO_2 progressively increases CBF [Paulson et al., 1990]. The effects of plasma glucose levels on the degree of ischaemic injury is also an important variable in experimental ischaemia [Chopp et al., 1988; Yip et al., 1991]. Hyperglycaemia facilitates acidosis and results in the exacerbation of the ischaemic injury [Chopp et al., 1988]. Alterations in the haematocrit results in either increasing viscosity of the blood, thus impaired flow through the capillary network or reduced oxygen carrying capacity inducing hypoxia to the target area. Brain temperature has in recent years become a controversial issue in experimental ischaemia. Investigations of Busto et al. (1987) have demonstrated that reductions in brain temperature by 4°C in transient global or focal ischaemia results in a substantial reduction in the size of infarct [Minanisawa et

al., 1990; Morikawa et al., 1992]. The regulation of rectal and brain temperature in experimental studies will facilitate the reproducibility of the insult [Ridenour et al., 1992; Busto et al., 1987; Minanisawa et al., 1990; Morikawa et al., 1992; Zhang et al., 1993]. The impact of MABP on CBF and ischaemic damage has been well documented. The autoregulatory capacity of cerebral vessels is impaired in ischaemia and CBF varies passively with alterations in MABP [Paulson et al., 1990]. Hypotension below the lower limit of autoregulation results in increase in infarct size following a focal ischaemic insult [Osborne et al., 1987]. Haemorrhagic hypotension is also used in transient forebrain ischaemia to exacerbate the extent of ischaemia [Smith et al., 1984]. Prolonged hypotension *per se* can result in ischaemic damage in the border zones of the three cerebral arteries.

The experimental models are important for the understanding to the mechanism associated with cerebral ischaemia and also for the development of therapeutic strategies for the amelioration of ischaemic damage.

Subdural Haematoma

Subdural haematomas are associated with ischaemic neuronal damage and high mortality. The neuronal damage may be ipsilateral to the haematoma or occasionally bilateral. Surgical intervention is the main course of treatment and ~50% of patients die and 25-25% of patients are left with disabilities [Sahuquillo-Barris, 1988]. The subdural haematoma is associated with increases in intracranial pressure, herniation of the brain and a reduction in cerebral perfusion pressure [Sahuquillo-Barris, 1988; Bullock & Teasdale, 1990].

The model of subdural haematoma used in the present investigation was developed in the rat [Miller et al., 1990]. Autologous venous blood (~400 μl) was injected slowly into the subdural space [Miller et al., 1990]. The volume of blood injected has been previously demonstrated to be the optimal amount necessary for the induction of consistent brain lesions [Miller et al., 1990]. The blood was injected over 5-7 min to allow for the formation and discrete location of the haematoma (a rapid injection of the blood into the subdural space did not result in the formation of a

haematoma) [Miller et al., 1990]. The slow injection of blood may be analogous to the clinical situation where there may be a slow leak of blood from the ruptured vessels into the subdural space [Gennarelli & Thibault, 1982].

The neuronal damage associated with the subdural haematoma is the result of a number of factors. The mass of the subdural haematoma may result in the herniation of the brain across the midline and increased pressure on the brainstem in humans. These alterations are associated with increases in intracranial pressure and acute swelling of the brain probably due to cytotoxic oedema. The brain is also vulnerable to the constituents of blood such as thrombin and platelet activating factor that facilitate the progression of the ischaemic injury. The presence of blood in the subdural space may trigger the release of vasoconstrictor agents such as 5-HT and endothelins. The constriction of arterioles on the surface may be a contributing factor to the development of the ischaemic lesion. The pattern of neuronal damage and CBF following subdural haematoma in the rat are dependent on the distribution of the clot with the greatest extent of ischaemic damage directly under the greatest mass of the haematoma and the alterations becoming more diffuse as the haematoma spreads over the convexity of the brain.

Focal Ischaemia

A focal ischaemic insult is one of the most common forms of stroke in man. The middle cerebral artery is the most commonly affected artery in man and this has led to the development of animal models where the patterns of ischaemic following MCA occlusion are modelled [Derouesue et al., 1993]. An experimental model of focal ischaemia was first described in the rat where the MCA was ligated [Robinson et al., 1975]. Neuronal damage was observed in the cerebral cortex but the size of the lesion was variable [Robinson et al., 1975]. Subsequently, Tamura and colleagues (1981a &b) developed a model of focal ischaemia in the rat which involved the permanent occlusion of the MCA and its lenticulostriate branches, using diathermy, and a lesion in the caudate nucleus and neocortex was obtained. In the rat occlusion of the MCA and its branches results in the ischaemic damage in the territory of the MCA

and the histologic changes associated with focal cerebral ischaemia are observed 3-4 hours following the insult [Tamura et al., 1981a & 1981b; Kaplan et al., 1991].

O'Brien and Waltz (1973) described a transorbital technique for the occlusion of the MCA in the cat. Permanent occlusion of the MCA and lenticulostriate branches results in an ischaemic lesion within the territory of the MCA. There are a number of advantages in using a larger animal species such as the cat over the rat. The cat is a gyrencephalic species, like man, and may be a more representative model for the processes underlying ischaemia [O'Brien & Waltz, 1973; Strong et al., 1983a & b]. Depolarisation caused by the efflux of potassium ions result in a wave of the depolarisation rapidly traversing the surface of the brain. The propagation of the waves of spreading depression following ischaemia are thought to exacerbate the extent of ischaemic damage. Actions of neuroprotective agents, such as MK-801, in the rat have been attributed to their ability to attenuate the waves of spreading depression. The stability of cardiovascular and respiratory parameters is of paramount importance in ischaemia studies. The cat has a greater stability under prolonged anaesthesia than the rat (over a period of 4-6 hours for acute studies). The larger blood volume in the cat allows greater cardiovascular stability under conditions of repeated sampling of blood for gases and plasma drug levels that are necessary in a focal cerebral ischaemia study.

The occlusion of the MCA in the rat and cat is an invasive procedure. The craniectomy and surgical exposure of the MCA involves a degree of local tissue damage. The larger brain size in the cat means that the damage caused by the exposure has a smaller contribution to the overall volume of the damage in the cat than in the rat. The neuroprotective drug strategies in focal ischaemia rely on the salvage of tissue in the peri-focal regions (penumbra) [Chen et al., 1991a, Mackay et al., 1993b; Bullock et al., 1994]. There is a greater distance of the penumbra from the site of surgery and a larger volume of salvageable tissue in the gyrencephalic species.

There is an increase in the number of anastomotic links between the cerebral arterioles [Mchedlishvili & Kuridze, 1984] and investigations of flow enhancing strategies will be facilitated in the larger animal species. The surgical manipulation of

the MCA may affect the vascular reactivity of vessels in more distal regions and the greater distance of the cerebral arterioles to be examined from the site of surgery would minimise such effects.

Global Ischaemia

Experimental models of global cerebral ischaemia have been developed to gain insight into the mechanism of brain damage associated with cardiac arrest. There are a variety of animal models of global cerebral ischaemia and the techniques involve the occlusion of arteries in the neck [Pulsinelli et al., 1979; Smith et al., 1984]. The species used for models of global cerebral ischaemia include dog, cat, gerbil and rat. The rat is most commonly used species and the models used are a four vessel occlusion model described by Pulsinelli et al. (1979) and a 2 vessel occlusion with haemorrhagic hypotension described by Smith et al (1984).

In the four vessel occlusion model, the vertebral arteries are cauterised and global cerebral ischaemia induced by ligation of the carotid arteries for between 5-30 min [Pulsinelli et al., 1979]. There is a marked reduction in CBF <5% of baseline during the ischaemic period and the release of the carotid ligatures results in a transient hyperaemic response followed by a period of delayed post-ischaemic hypoperfusion [Pulsinelli et al., 1979] (Figure 4). In the two vessel occlusion model, the carotid arteries are ligated for 5-20 min and haemorrhagic hypotension induced [Smith et al., 1984]. The occlusion of the carotid arteries results in a moderate reduction in CBF and the induction of haemorrhagic hypotension is necessary to induce cerebral ischaemia [Smith et al., 1984]. The release of the carotid ligatures and restoration of mean arterial blood pressure results in similar alterations in CBF [Smith et al., 1984].

The interest in global ischaemia has been concentrated on the patterns of selective neuronal vulnerability [Schmidt-Kastner & Freund, 1991]. The neuronal damage is observed primarily in the CA1 region of the hippocampus, neocortex and some damage in the corpus striatum [Kirino et al., 1984; Schmidt-Kastner & Freund, 1991]. Additionally, selective neuronal vulnerability observed in the hippocampus is

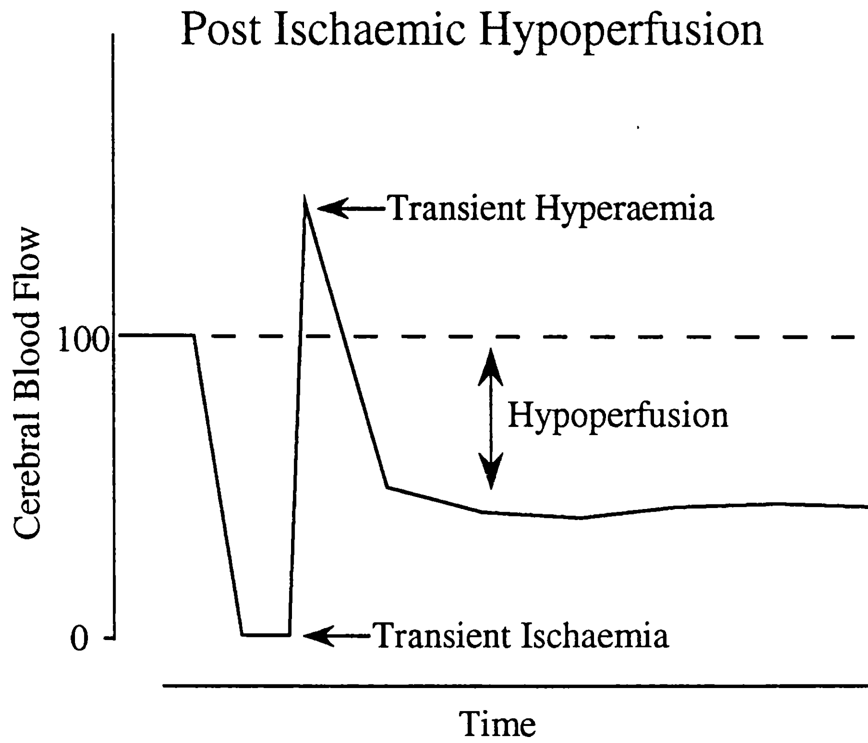


Figure 4 Schematic diagram illustrating the transition from hyperaemia to hypoperfusion following transient global cerebral ischaemia

observed in humans following cardiac arrest [Brierley & Graham, 1984; Petito et al., 1987].

Delayed post-ischaemic hypoperfusion observed following global or forebrain ischaemia has been attributed to a number of factors [see discussion]. Manipulations with calcium channel antagonists have indicated that the maintenance of post-ischaemic hypoperfusion may be the result of a loss of dilator tone or the release of vasoconstrictor factors [Kazda et al., 1982].

The availability of animals models of cerebral ischaemia allows us address a number of issues. The rigorous control of cardiovascular and respiratory parameters facilitates the understanding of the mechanisms of ischaemic neuronal damage. Animal models of cerebral ischaemia made important contributions in the development of therapeutic agents for the amelioration of ischaemic damage.

1.3 Endothelin: a vasoconstrictor peptide

Discovery

The vascular endothelium is the source of many vasoactive factors. Recent research on the vascular endothelium has shed light on the multiple roles of the endothelins in the regulation of vascular tone. The main factors that have been isolated are the vasodilator substances endothelium derived relaxing factor (EDRF), prostacyclin and endothelium derived contracting factor (EDCF) [Moncada et al., 1976; Furchgott & Zawadski, 1980; Hickey et al., 1985]. The importance of the role of EDRF and prostacyclin in the maintenance of vascular tone has been well documented. EDRF has subsequently been suggested to nitric oxide or a nitrosothiol. However, the identification and role of EDCF has proved to be elusive.

The initial existence of an endothelium derived vasoconstrictor factor was suggested. The discovery of the EDCF was facilitated by the discovery of a vasoconstrictor substances from endothelial cell cultures by Hickey et al. (1985). The culture medium from aortic endothelial cells triggered a vasoconstrictor response that developed slowly but had a prolonged duration of action. These actions could not be attributed to any of the known vasoconstrictors and additionally it was demonstrated that this vasoconstrictor factor was of peptidergic origin. These observations were subsequently repeated by various other groups.

In 1988 Yanagisawa and colleagues published a seminal paper describing the isolation, cloning and sequencing of a peptidic EDCF that they termed endothelin (ET). The endothelin peptide was isolated initially from bovine aortic endothelial cell cultures. The effects of this peptide were similar to effects described by Hickey and colleagues, i.e., slow developing contraction and prolonged duration of action. The endothelin peptide described by Yanagisawa and colleagues was a 21 amino acid peptide and there appeared to be no sequence homology with any other peptides of mammalian origin. Subsequently a peptide isolated from snake venom (*Atracaspis engaddensis*, Israeli burrowing asp) was described to have a structure very similar to the endothelins.

Structure

The endothelin family of peptides comprises three isopeptides called endothelin-1 (ET-1), endothelin-2 (ET-2) and endothelin-3 (ET-3). These peptides are encoded by three distinct genes and sequences for these have been previously published [Yanagisawa et al., 1988]. The three isopeptides share large degrees of sequence homology and the mature peptides consist of 21 amino acids with the distinguishing feature of double disulphide bridges between cysteine residues at positions 1 - 15 and 3 -11. The differences between the amino acid sequences of ET-1, ET-2 and ET-3 are shown in figure 5.

Synthesis

The processing of pre pro endothelin peptide is carried out in 3 stages. The first stage involves the removal of the signal peptide by a dibasic peptidase to give a pre pro endothelin of 212 amino acid residues. The second stage involves the formation of pro endothelin or big endothelin (41 amino acid residues) by a carboxy peptidase [Doherty, 1992; Battistini et al., 1993; Rubanyi & Polokoff, 1994]. The final stage is the production of the 21 amino acid mature endothelin peptide by the endothelin converting enzyme [Opgenorth et al., 1992; Schmidt et al., 1995; Deschepper et al., 1995] [Figure 6]. The conversion of the big-ET form to the mature endothelin peptide is an important phase because the vasoconstrictor activity of the big-ET form is approximately 100 fold less than the endothelin.

Synthesis of the mature endothelin peptides (s) relies on their conversion from the big endothelin form. This processing is carried out by an enzyme putatively termed endothelin converting enzyme (ECE). The initial descriptions of this enzyme indicated that it may be a chymotrypsin like enzyme (serine protease). However, serine proteases inhibitors failed to inhibit the vasoconstriction actions of BET-1. Further analysis demonstrated two protease enzymes, an aspartic protease from bovine and porcine aortic endothelial cell cultures, bovine adrenal medulla and rat lung, and a metalloprotease isolated from bovine and porcine aortic endothelial cell cultures, porcine vascular smooth muscle cells and from human and rat brain. These enzymes

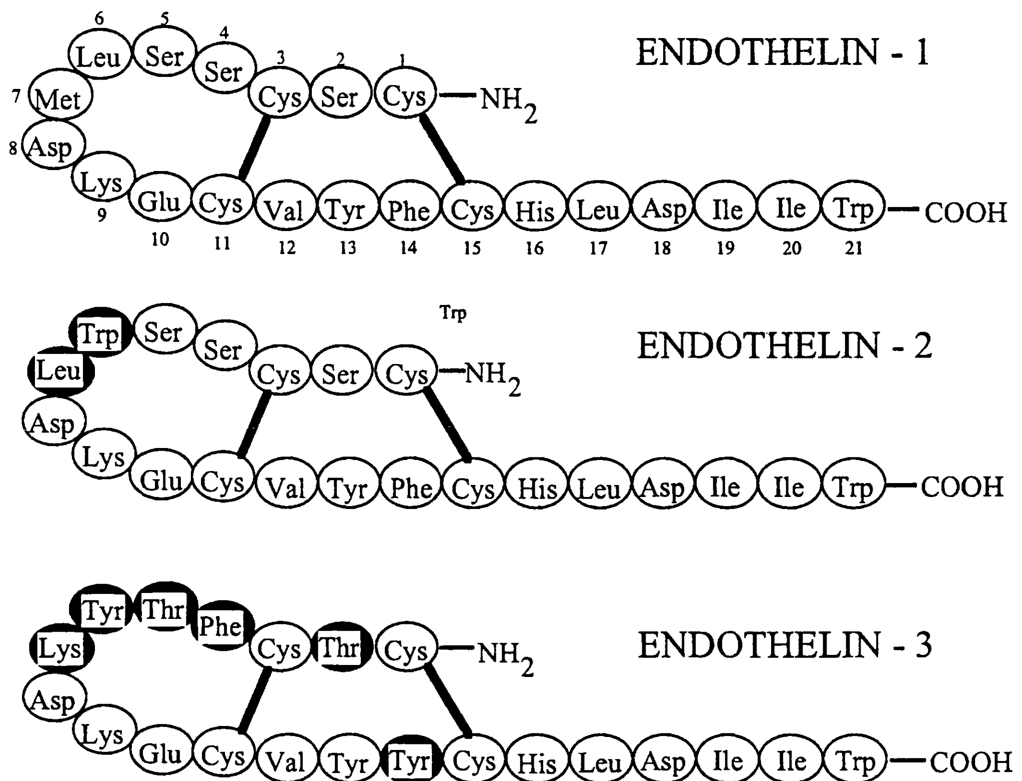


Figure 5 The structure of the mature endothelin peptides. The 21 amino acid peptide molecules are characterised by the two disulphide bridges between the cysteine amino acids present at 1, 3, 11, 15. The shaded amino acids (black) indicate the differences between ET-2, ET-3 and ET-1.

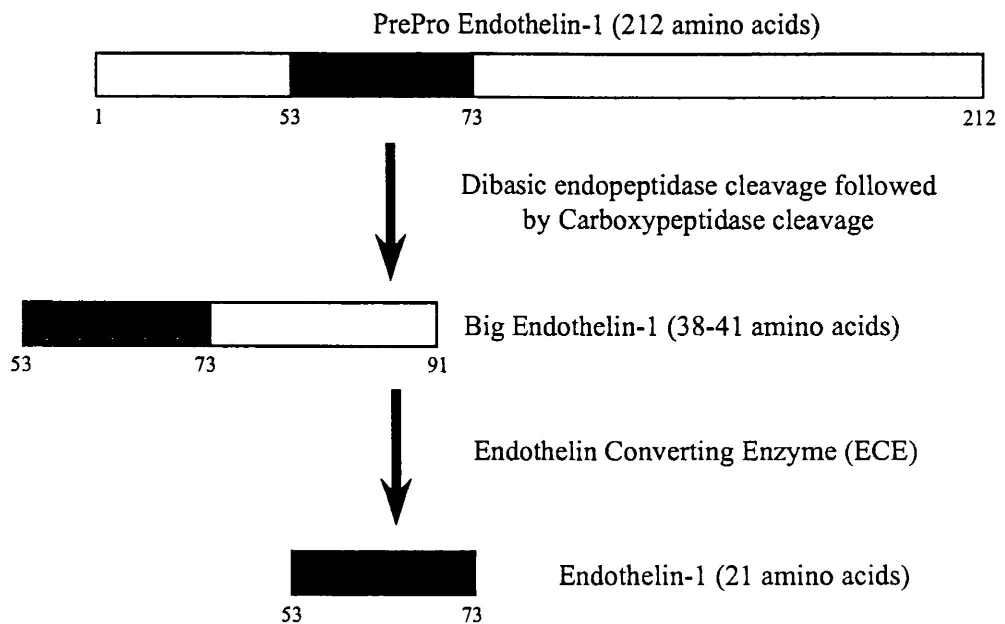


Figure 6 The processing of the prepro-endothelin peptide to the mature endothelin peptide. The processing is facilitated bya carboxypeptidase and Endothelin Converting Enzyme (ECE). ECE is a metalloprotease and specific ECE's mayinvolved in the processing of ET-1, ET-3 and ET-3.

functioned at different optimal pH (pH 5.5 for the aspartic protease and pH 7.4 for the metalloprotease protease). These observations suggested that there may be 2 or more forms of ECE and they may be involved in the conversion of BET-1 to ET-1 in endothelial cells or on vascular smooth muscle cells because of its optimal pH. The metalloprotease is more commonly known as the Neutral Endopeptidase (NEP 24.11) and facilitates the conversion of BET-1 to ET-1 and is inhibited by phosphoramidon while thiorphan (also an NEP inhibitor) had no effect on BET-1 conversion. The acidic protease is inhibited by pepstatin. Phosphoramidon prevented the conversion of BET-1 released from endothelial cells to ET-1. The aspartyl protease inhibitors had no effect on BET-1 conversion and hence the NEP form was thought to be physiologically relevant. Additionally, phosphoramidon reduced the MABP in SHR rats and inhibited the vasoconstrictor effects of BET *in vivo*. The phosphoramidon sensitive ECE was identified in the membrane fraction in porcine aortic endothelial cells. The activity of the membrane fraction was not influenced by thiorphan and the cytosolic was insensitive to phosphoramidon. ECE activity in vascular smooth muscle cells have demonstrated similar acidic and neutral and pH forms. Since the release of endothelin (BET) is polarised towards the vascular smooth muscle, the presence of ECE on these surfaces maybe involved in the conversion to mature ET peptide.

Paradoxically, NEP 24.11 (enkephalinase) has been described as the enzyme involved in the degradation of the endothelin peptide amongst other biological peptides such as ANP, enkephalins, bradykinin and substance P. NEP is also inhibited by phosphoramidon and physiologic ECE may be an NEP-like enzyme.

Endothelin Receptors and Functions

The isolation of the endothelin isopeptides was rapidly followed by the cloning and identification of cDNA encoding two endothelin receptors, ET_A and ET_B [Arai et al., 1990; Sakurai et al., 1990; Masaki et al., 1994]. The pharmacologic characterisation of these receptors was carried out on the basis of the rank order of potencies of the endothelin isopeptides. The endothelin ET_A receptor showed increased selectivity for ET-1 and ET-2 over ET-3 [Arai et al., 1990]. The three

isopeptides are equipotent at the ET_B receptor [Sakurai et al., 1990]. A receptor displaying increased selectivity for ET-3 over ET-1 or ET-2 has been described in amphibian melanocytes [Karne et al., 1993]. This receptor has putatively been called the endothelin ET_C receptor, however, a mammalian correlate of this receptor has not been isolated or cloned.

There is a high degree of sequence homology in the structure of endothelin receptors (approximately 50% of amino acids in a variety of species), similar to endothelin peptides [Arai et al., 1990; Sakurai et al., 1990]. The endothelin receptors belong to the rhodopsin superfamily of G-protein linked receptors. The two receptor subtypes have seven transmembrane spanning domains that is characteristic of this family.

These contractile actions are meditated by the increase in intracellular calcium and there are a number different mechanisms that can facilitate this increase in intracellular calcium. These mechanism include G-protein activated phospholipase C (PLC) resulting in an increase in phosphotidyl inositol hydrolysis (PI hydrolysis) and production of diacyl glycerol (DAG), the activation of protein kinase C (PKC) by DAG, activation of phospholipase A_2 (PLA_2) and arachidonic acid metabolism and activation of phospholipase D (PLD) [Rubanyi & Polokoff, 1994]. The increases in intracellular calcium results in the activation of the contractile mechanism of the smooth muscle and the contraction of the vasculature and other smooth muscle tissues [Rubanyi & Polokoff, 1994; Tamirisa et al., 1995].

The vasoconstrictor effects of the endothelins are primarily mediated via the endothelin ET_A receptor [Doherty, 1992; Rubanyi & Polokoff, 1994; Gandhi et al., 1994]. Vasodilatation is mediated by ET_B receptors *in vivo* and *in vitro* [Rubanyi & Polokoff, 1994; Fukuroda et al., 1990; Clozel et al., 1992; Shetty et al., 1993], however, ET_B receptor mediated vasoconstrictions have been described in some vascular beds e.g. kidney and pulmonary circulation [Rubanyi & Polokoff, 1994; Clozel et al., 1992; Shetty et al., 1993; Seo et al., 1994]. The pulmonary vasculature demonstrates a vasoconstrictive response that is mediated via both endothelin ET_A and endothelin ET_B receptors [Rubanyi & Polokoff, 1994; Warner et al., 1993; Bax et al.,

1993; Fukuroda et al., 1994; Cardell et al., 1993]. Smooth muscle from the guinea pig fundus and small intestine in demonstrate endothelin ET_A receptor mediated dilatation [Lin & Lee, 1990 & 1992]. The detailed effects of the endothelins in the periphery have been reviewed elsewhere [Rubanyi & Polokoff, 1994; Gandhi et al., 1994]. The varied effects of the endothelins at the ET_B receptor have led to the subdivision of the ET_B receptor into ET_{B1} receptors that mediate vasodilatation and ET_{B2} receptors that mediate vasoconstriction [Masaki et al., 1994; Warner et al., 1993].

Endothelin-1 elicits a vasoconstriction of vessels in the renal, mesenteric and coronary circulations [Clozel et al., 1989; Gellai et al., 1994; Wallace et al., 1989]. The intravenous administration of endothelin-1 effects a transient decrease followed by a sustained increase in mean arterial blood pressure. The available evidence indicates that the endothelin ET_A receptors mediate the vasoconstrictor response in blood vessels and are located on the vascular smooth muscle [Rubanyi & Polokoff, 1994; Masaki et al., 1994]. The vasoconstrictor effects of endothelin-1 are calcium dependent and are attenuated by the calcium channel antagonists nitrendipine and nifedipine [Rubanyi & Polokoff, 1994; Godfraind et al., 1989; Egashima et al., 1990; Encabo et al., 1992]. The transient decrease in blood pressure has been attributed to endothelial ET_B receptor activation and is a result of NO or PGI_2 release [Fozard & Part, 1992; Ishikawa et al.., 1994; Hirata et al., 1993].

Endothelin Receptors in Cerebral Vessels

The potent vasoconstrictor actions of exogenous endothelin-1 have been demonstrated in isolated cerebral arteries and arterioles in a variety of species [Hardebo et al., 1989; Saito et al., 1989 & 1991] (see Table 13 in section 4.1). The vasoconstrictor actions of endothelin-1 have also been demonstrated on cerebral resistance arterioles and intracerebral arterioles *in vivo* [Salom et al., 1995; Willette & Sauermelch, 1990; Faraci, 1989; Robinson & McCulloch, 1990]. The systemic administration of exogenous endothelin demonstrated alterations in cerebral blood flow. However, the alterations in cerebral blood flow were related to the increases in

mean arterial blood pressure and not the result of direct effect on the cerebrovasculature. Systemic exogenous endothelin does not gain access to the abluminal surface of the cerebrovasculature. The in vitro investigations of Saito and colleagues (1991) demonstrated that endothelin exerts its contractile effects following its abluminal application. The release of endogenous endothelins is polarised towards the cerebrovascular smooth muscle [Yoshimoto et al., 1990; Wagner et al., 1992; Mima et al., 1989] and this is thought to be the location of the constrictor endothelin ET_A receptors [Takayanagi et al., 1990; Davenport et al., 1990]. Transient vasodilator effects have been demonstrated in isolated cerebral arteries and the effects have been attributed to endothelin ET_B receptor activation [Salom et al., 1995].

The role of the endothelins in the regulation of cerebrovascular tone and CBF remains controversial. Investigations in endothelial and vascular smooth muscle cell cultures have indicated that there may be a basal release of endothelin-1 which may be involved in the regulation of vascular tone [Yoshimoto et al., 1990; Kobayashi et al., 1991; McCarron et al., 1993; Durieu-Trautmann et al., 1993]. However, other investigations *in vivo* have indicated that there is minimal basal endothelin mediated tone (demonstrated by the non-effect of endothelin receptor antagonists) and endothelin may not be involved in the regulation of arterial or arteriolar calibre or CBF. (See Section 4.1 for current concepts in endothelin receptor classification in the cerebrovasculature)

Endothelin Receptors in the Central Nervous System

Endothelin receptor binding sites have been described in glial cells (astrocytes) and neurones (e.g. dorsal root ganglia) [Niwa et al., 1992; Jones et al., 1989; Giaid et al., 1989; MacCumber et al., 1990; Greenberg et al., 1992; Koseki et al., 1989]. Binding studies with radiolabelled endothelin-1 have indicated the presence of binding sites in the cerebellum, cerebral cortex, dentate gyrus in the hippocampus and in nuclei in the brainstem [Jones et al., 1989; Kurihara et al., 1990; Nambi et al., 1990]. The indications are that the predominant receptor subtype is the endothelin ET_B receptor [MacCumber et al., 1990; Nambi et al., 1990; Vigne et al., 1991]. The

presence of ET mRNA has been described in glial cells (astrocytes) and neurones (e.g. dorsal root ganglia) [Niwa et al., 1992; Jones et al., 1989; Giaid et al., 1989; MacCumber et al., 1990]. Early investigations provided evidence for ET mRNA in cells of the hypothalamus, striatum and also the spinal cord [Giaid et al., 1989; Lee et al., 1990]. Secretion of endothelin-1 by astrocytes has been reported and can be stimulated by thrombin and platelet derived growth factor (PDGF) [MacCumber et al., 1990; Lysko et al., 1991; Marsault et al., 1990; Levin et al., 1992]. The role of endothelins in the CNS is unclear, but, stimulation of immediate early gene expression (c-fos) and nerve growth factor in astrocytes indicates that these peptides may play a role in the regeneration of nerve cells following a cerebrovascular injury [Ladenheim et al., 1993].

A number of investigators have performed functional studies to examine the effects of exogenous endothelins in the brain. Injections of endothelin-1 into the cisterna magna or microinjections into discrete brainstem nuclei in the rat have elicited marked alterations in cardiovascular parameters with systemic hypertension and bradycardia being the most prominent [Siren et al., 1989; Ferguson & Smith, 1990; Macrae et al., 1991]. The measurement of CBF in the rat brainstem following an intracisternal injection of endothelin-1 demonstrated a marked reduction in CBF to pathological levels [Macrae et al., 1991]. Other investigations have indicated that the endothelins may play a role as neuromodulators in the locomotor system or in the release of pituitary hormones [Gross et al., 1993 & 1994]. The intracerebroventricular injection of endothelin-1 elicited behavioural effects such as barrel rolling and oculo-clonic seizures [Gross et al., 1993 & 1994] and are associated with alterations in CBF and cerebral glucose utilisation in peri-ventricular regions and areas distal to the site of injection [Gross et al., 1993 & 1994]. These observations suggest a role for the endothelins as neuromodulators or neuropeptides [Gross et al., 1994; Berrino et al., 1994]. The alterations in CBF and glucose utilisation were attenuated by the calcium channel and endothelin antagonists. However, the effects of endothelin receptor antagonists *per se* on neuronal function has not been examined.

Endothelin Receptor Antagonists

The development of endothelin receptor antagonists was important for understanding the role of endothelins in vascular pathophysiology. The first antagonists described were natural by-products of the fermentation of *Streptomyces misakiensis* (BE-18257B) [Ihara et al., 1991]. This compound demonstrated low potency at endothelin receptors in binding and functional bioassays (IC_{50} ET_A 1.4μM; ET_B >100μM) [Ihara et al., 1991]. Structure activity relationship studies have led to modifications of the peptide structures of the endothelins and to the development of a series of peptide endothelin ET_A, endothelin ET_B and combined endothelin ET_A/ET_B endothelin receptor antagonists [Ishikawa et al., 1994; Ihara et al., 1992a; Cody et al., 1993; Itoh et al., 1993] [Table 2]. BQ-123 is the most potent of this series and is selective for the endothelin ET_A receptor [Ihara et al., 1992a].

The distinguishing feature of the cerebral circulation is the blood-brain barrier. The blood-brain barrier is formed of tight junctions between endothelial cells and prevents paracellular transport of large and charged molecules [Edvinsson et al., 1993]. The absence of pinocytotic vesicles prevents transcellular transport of molecules across the blood-brain barrier [Ermisch et al., 1993]. The presence of the blood-brain barrier means that therapeutic agents, such as the endothelin receptor antagonists, that are targeted at the brain must penetrate this barrier to gain access to the cerebrovascular smooth muscle and neurones. There are a number of disadvantages in the use of peptide endothelin receptor antagonists as therapeutic agents for cerebrovascular diseases. The peptide antagonists would undergo rapid hydrolysis by peptidases present in the systemic circulation or in the gastro-intestinal tract if administered intravenously or orally. More importantly, the peptide endothelin antagonists do not penetrate the blood-brain barrier when administered systemically [Clozel et al., 1993]. The importance of blood-brain barrier permeability for endothelin antagonist was demonstrated by the failure of intravenous administration of peptide endothelin receptor antagonist (BQ-123) to attenuate cerebral vasospasm or vasoconstrictor action of exogenous endothelin-1 applied to cerebral arterioles [Clozel & Watanabe, 1993].

Table 2

Endothelin receptor agonists and antagonists used in cerebrovascular research. Data are expressed as the concentration of drug that inhibited binding of [^{125}I]-endothelin-1 to endothelin-A (ET_A) and endothelin-B (ET_B) receptors by 50% (IC_{50}). Abbreviations indicate the tissue used in the assays.

Compound	Action	ET_A (nM)	ET_B (nM)	Reference
Peptide molecules				
ET_A selective				
BQ123	Antagonist	22 (PVSM)	1800 (PCM)	Ihara et al., 1992
BQ485	Antagonist	3.4 (PVSM)	26000 (PCM)	Itoh et al., 1993
FR139317	Antagonist	1 (HR)	7300 (HR)	Sogabe et al., 1993
ET_B selective				
BQ3020	Agonist	940 (PVSM)	0.2 (PCM)	Ihara et al., 1992
IRL1620	Agonist	0.016 (PLM)	0.2 (PLM)	Tanaki et al., 1992
BQ788	Antagonist	280 (PCA)	0.9 (PCM)	Ishikawa et al., 1994
IRL1038	Antagonist	9 (RLM)	700 (RAM)	Urade et al., 1992
Combined ET_A/ET_B				
PD142893	Antagonist	40 (CRRA)	40 (RCM)	Cody et al., 1993
PD145065	Antagonist	3.5 (CRRA)	15 (RCM)	Cody et al., 1993
TAK044	Antagonist	3.8 (RVM)	130 (RBCM)	Watanabe et al., 1995
Non-peptide molecules				
ET_A selective				
PD155080	Antagonist	7.8 (HR)	3500 (HR)	Doherty et al., 1995
PD156707	Antagonist	0.31 (HR)	420 (HR)	Doherty et al., 1995
BMS182874	Antagonist	48 (HR)	>50000 (HR)	Webb et al., 1995
Combined ET_A/ET_B				
Bosentan	Antagonist	4.7 (HR)	95 (HR)	Clozel et al., 1994
Ro46-2005	Antagonist	220 (HVSM)	1000 (RAEC)	Clozel et al., 1993
L754142	Antagonist	0.06 (PVSM)	2.2 (PCM)	Williams et al., 1995
L751281	Antagonist	0.64 (PVSM)	13.1 (PCM)	Walsh et al., 1995
SB209670	Antagonist	0.22 (HR)	11 (HR)	Nambi et al., 1994
SB217242	Antagonist	1.1 (HR)	111 (HR)	Lago et al., 1995

Abbreviations: **CRRA** = Cultured Rabbit Renal Artery Vascular Smooth Muscle Cells; **HR** = Human Receptors expressed in Chinese Hamster Ovary cells; **HVSM** = Human Vascular Smooth Muscle Cells; **PCA** = Porcine Coronary Artery Smooth Muscle Cells; **PCM** = Porcine Cerebellar Membrane; **PLM** = Porcine Lung Membranes; **PVSM** = Porcine Vascular Smooth Muscle; **RAEC** = Rat Aortic Endothelial Cells; **RAM** = Rat Aortic Membranes; **RCM** = Rabbit Cerebellar Membranes; **RCM** = Rat Cerebellar Membranes; **RLM** = Rat Lung Membranes; **RVM** = Rabbit Ventricular Membranes

Recently there has been the development of non-peptide endothelin receptor antagonists [Table 2]. The first non peptide endothelin receptor antagonist described was Ro46-2005 and its therapeutic potential was demonstrated in experimental subarachnoid haemorrhage [Clozel et al., 1993b]. A number of other potent non-peptide endothelin receptor antagonists selective for the endothelin ET_A and combined endothelin ET_A/ET_B receptors have been developed. We have examined the ability of non-peptide endothelin receptor antagonists to gain access to the cerebrovascular smooth muscle and attenuate the vasoconstriction induced by the adventitial application of endothelin-1 and demonstrated the potential of these antagonists as therapeutic agents. However, the non-peptide structure of an endothelin receptor antagonist does not necessarily confer blood-brain barrier permeability (e.g. SB209670) [Ohlstein et al., 1994].

The attenuation of the effects of endogenous endothelins in cerebrovascular disease may also be achieved by endothelin converting enzyme inhibitors. The cDNA encoding endothelin converting enzyme(s) (ECE) have recently been cloned and will facilitate the development of enzyme inhibitors selective for ECE [Deschepper et al., 1995; Schmidt et al., 1995]. In contrast to the rapid development of endothelin receptor antagonists, only a few ECE inhibitors have been developed e.g. Phosphoramidon, CGS26303, CGS26393 [Fukami et al., 1994; De Lombaert et al., 1995]. The utility of the ECE inhibitors has been examined in preliminary studies in focal cerebral ischaemia [Duverger et al., 1993]. (See Section 4.1.2 for description of effects of endothelin receptor antagonists in the cerebrovasculature)

Pathophysiological Role of Endothelins

The potent constrictor actions of endothelins have implicated them in a variety of vascular and non vascular pathologies [Rubanyi & Polokoff, 1994]. There is evidence for a role of the endothelins in cardiac ischaemia, renal ischaemia and hypertension [Rubanyi & Polokoff, 1994] (see Table 3).

The effects of the endothelins were initially examined in isolated blood vessels and their effects on mean arterial blood pressure. The intravenous infusion of ET-1

Table 3

Putative Role of Endothelins in Pathophysiology

- Hypertension
- Pulmonary Hypertension
- Renal Hypertension
- Cardiac Ischaemia
- Congestive Heart Failure
- Renal Ischaemia
- Bronchoconstriction
- Subarachnoid Haemorrhage
- Stroke

elicited a biphasic response on mean arterial blood pressure, an initial transient vasodilatation followed by a prolonged and sustained vasoconstriction [Yanagisawa et al., 1988; De Nucci et al., 1988]. Subsequent investigations using endothelin receptor subtype selective antagonists have identified the vasodilator response being mediated by endothelin ET_B receptors localised on the vascular endothelium and the release of nitric oxide [Fozard & Part, 1992; Bazil et al., 1992]. The dilator responses were inhibited by the endothelin ET_B receptor selective antagonists and the nitric oxide synthetase inhibitors [Fozard & Part, 1992; Bazil et al., 1992; Ishikawa et al., 1994]. The endothelin ET_A receptors have been localised to the vascular smooth muscle. The rank order of potency of the endothelin receptor agonists for mediating the pressor responses were ScTx 6b>ET-1>>ScTx 6c> ET-3. This indicated an endothelin ET_A receptor mediated response. The vasoconstrictor actions were attenuated by the endothelin ET_A receptor antagonists e.g. BQ-123 [Bazil et al., 1992; Ihara et al., 1992; McMurdo et al., 1992]. The contractile agents 5-HT and noradrenaline potentiated the vasoconstrictor actions of ET-1 and ET-1 potentiated the actions of 5-HT and noradrenaline [Tabuchi et al., 1989; Yang et al., 1990].

In cardiac tissue, ET-1 production was observed in cultured endocardial cells [Siney & Lewis, 1994]. Additionally, the mRNA for pre pro endothelin-1 was identified in rat cardiac myocytes [Rubanyi & Polokoff, 1994]. Receptor binding sites specific for endothelins were identified in cardiac myocytes, cardiac membranes and in the atrio-ventricular node [Hirata et al, 1989; Gu et al., 1990; Yamasaki et al., 1989]. Messenger RNA for the endothelin ET_A/ET_B receptor subtypes were localised in the atrial and ventricular myocardium [Molenaar et al., 1993]. In isolated perfused hearts, ET-1 induced a positive chronotropic and inotropic effect and increased the duration of the cardiac action potential [Firth et al., 1990; Watanabe et al., 1989]. Exogenous ET-1 induced coronary vasoconstriction and a reduction in cardiac function. The positive inotropic effect elicited by ET-1 was observed in isolated perfused hearts, atria and ventricular cells from a variety of species [Rubanyi & Polokoff, 1994]. In isolated rat hearts ET-1 induced a dose dependent increase in the heart rate [Ishikawa et al., 1988]. The positive chronotropic effect was delayed in

onset and had a prolonged duration of action [Ishikawa et al., 1988]. The intravenous injection of ET-1 induced bradycardia, although, the bradycardic responses were thought to be secondary to the reduction in coronary blood flow [Lerman et al., 1992].

Exogenous endothelin-1 is potent constrictor of the coronary circulation. The constriction of the coronary vessels results in a reduction in coronary blood flow and may even result in the occlusion of the coronary arteries [Kurihara et al., 1989; Clozel & Clozel, 1989; Hom et al., 1992]. Exogenous endothelin-1 induces myocardial ischaemia and ventricular fibrillation in the canine heart [Hori et al., 1991; Muramatsu et al., 1991]. A reduction in cardiac output has been observed by some authors but the effect may be related to the coronary vasoconstriction. The actions of ET-1 in cardiac myocytes have been related to increases in $[Ca^{2+}]_i$, a result of increased hydrolysis of phosphotidyl inositol [Kelly et al., 1990].

Cardiac ischaemia has been another area where there has been speculation about the role of the endothelins [Watanabe et al., 1990]. The available evidence has indicated that there is an increased synthesis and release of ET-1 and elevated plasma ET-1 levels associated with cardiac ischaemia/hypoxia [Brunner et al., 1992; Rubanyi & Polokoff, 1994]. The increased binding of ET-1 to ischaemic tissue and increased reactivity of coronary blood vessels to ET-1 during ischaemia and reperfusion has further fuelled speculation [Rubanyi & Polokoff, 1994]. Antibodies raised against endothelin, endothelin receptor antagonists and the ECE inhibitor phosphoramidon have been demonstrated to reduce myocardial ischaemic damage [Grover et al., 1992; Watanabe et al., 1990].

There is an increase in the endothelin-1 induced coronary vasoconstriction following coronary artery occlusion and reperfusion [Neubauer et al., 1991]. Exogenous ET-1 is a pro-arrhythmic agent in the canine myocardium *in vivo* and *in vitro* [Muramatsu et al., 1991; Hom et al., 1992; Salvati et al., 1991]. Increased binding of ET-1 to cardiac membranes and increased synthesis/ release of ET-1 has been demonstrated following ischaemia and reperfusion in the rat and canine heart [Liu et al., 1990; Nayler et al., 1992; Neubauer et al., 1991]. The intracoronary infusion of ET-1 induced myocardial ischaemia while the topical administration of

ET-1 on the myocardium in the canine caused a localised ischaemia [Grover et al., 1992; Hori et al., 1991]. The intracoronary infusions of ET-1 are associated with reductions in coronary blood flow, disturbances in the A-V node and ventricular fibrillation [Muramatsu et al., 1991].

Cardiac ischaemia and reperfusion can increase ET-1 production and increase the size of infarct [Velasco et al., 1993]. The responses may the result of an increased ET-1 production as much as the loss of dilator tone (endothelium damage and loss of nitric oxide release) [Neubauer et al., 1991; Saito et al., 1992]. The alterations in endothelin binding to the ischaemic myocardium have generated mixed results. Some authors have reported elevations in the binding of ET-1 to cardiac membranes while others have reported the absence of such changes [Liu et al., 1990; Neubauer et al., 1991; Nayler et al., 1992]. In some studies in cardiac ischaemia and reperfusion, a reduction of ET-1 production during the ischaemic period was reported while an increase was reported during the reperfusion phase [Brunner et al., 1992]. In patients with myocardial ischaemia a seven fold increase in plasma ET-1 levels and in some cases elevations in urine ET-1 levels have been reported [Watanabe et al., 1990 & 1991].

In experimental and clinical investigations elevations in plasma ET levels were reported in patients with congestive heart failure [Cody et al., 1993; Wei et al., 1994]. The reasons for the elevations in plasma ET levels were unclear but a reduction in the clearance of ET-1 from the lungs and increased production of ET-1 were two suggested causes [Cavero et al., 1990; Resink et al., 1990]. The endothelin receptor antagonist FR139317 reduces systemic MABP in a canine model of congestive heart failure and improves outcome [Clavell et al., 1995]. In patients bosentan has been demonstrated to exert similar effects. Arginine vasopressin and angiotensin II stimulate the release of ET-1 *in vitro*. In congestive heart failure the circulating levels of AVP and angiotensin II are elevated and they may stimulate the release of the endothelins [Kiowski et al., 1995].

ET-1 is also a potent constrictor of isolated blood vessels. The prolonged vasoconstriction in isolated blood vessels are antagonised by the endothelin ET_A

receptor antagonists indicating endothelin ET_A receptor mediated events [Rubanyi & Polokoff, 1994]. Recent investigations have demonstrated the existence of endothelin ET_B receptor mediated vasoconstriction in the pulmonary, renal and coronary circulations in some species [Cardell et al., 1992; Warner et al., 1992; Shetty et al., 1993]. The investigations also alluded to the greater sensitivity of veins than arteries to the constrictor effect of ET-1 [Rubanyi & Polokoff, 1994]. In the lungs, the pulmonary arteries are more sensitive that the pulmonary veins to the constrictor actions of ET-1 [Cardell et al., 1990]. The reasons for these observations are unclear but *in vitro* investigations have demonstrated an increased sensitivity of pulmonary arteries to ET-1 following hypoxia. Isolated microvessels and resistance arterioles from a variety of vascular beds, including the skin, kidney, heart, gastric mucosa and lung, demonstrated vasoconstriction following exposure to ET-1 [Gandhi et al., 1994; Rubanyi & Polokoff, 1994]. The constrictor action of ET-1 could be reversed by the prostanoid PGI_2 and L- type calcium channel antagonists (verapamil, nifedipine, nitrendipine) [Rubanyi & Polokoff, 1994]. In hypertensive strains of rats and models of hypertension, ET-1 was a more potent constrictor of microvessels than in the normotensive animals. The elevation in MABP was mediated by the endothelin ET_A receptors.

ET-1/ET-3 stimulated the release of EDRF from bovine endothelial cell cultures and in blood vessels [Emori et al., 1991a, Warner et al., 1992; Namiki et al., 1992]. Endothelium dependent relaxations elicited by ET-1 could not be attenuated by the endothelin ET_A receptor antagonists [Rubanyi & Polokoff, 1994]. However, the combined endothelin ET_A/ET_B receptor antagonists and NOS inhibitors attenuated the vasodilator responses elicited by ET-1 [Gandhi et al., 1994; Rubanyi & Polokoff, 1994]. ET-1/ET-3 also stimulated the release of PGI_2 from human and bovine vascular endothelial cell cultures [Emori et al., 1991b; D'Orleans-Juste et al., 1992]. PGI_2 mediated vasodilatation elicited by ET-1/ET-3 has been demonstrated *in vivo* and *in vitro*.

The importance of peripheral vasoconstriction in the development of hypertension and the potent vasoconstrictor actions of ET-1 has formed the rational

basis of these conclusions. The increased release of ET-1 has been identified in animal models of hypertension but increases in circulating plasma levels have not been identified [Battistini et al., 1993]. In patients with essential hypertension, some authors have reported elevations in plasma ET-1 levels while others have suggested that no such increases are measurable [Davenport et al., 1991; Saito et al., 1991]. Most investigations measuring plasma ET-1 levels have used radioimmunoassay and the cross-reactivity of the antibodies with the other endothelin isopeptides and may account for the discrepancies. More importantly, there is increasing evidence that the release of endothelins is polarised towards the basement membrane and vascular smooth muscle [Wagner et al., 1992]. Thus, the concentrations of ET-1 detected in the circulation would not reflect the local concentrations which may be higher by a few orders of magnitude.

In other forms of hypertension such as malignant hypertension, pre-eclampsia, arteriosclerosis and renal hypertension, elevated plasma ET-1 levels have been detected [Battistini et al., 1993; Rubanyi & Polokoff, 1994]. The observations that blood vessels from hypertensive animals display a reduced responsiveness to ET-1 can be explained by receptor tachyphylaxis or downregulation of receptor numbers [Dohi & Luscher, 1991; Hollenberg et al., 1993]. There is evidence that endothelium dependent vasodilatation may be impaired under conditions of hypertension and this may enhance the vasoconstrictor effect of ET-1 [Dohi & Luscher, 1991; Luscher et al., 1991; Tamirisa et al., 1995]. The most controversial aspect of the role of endothelins in hypertension is the effects of endothelin receptor antagonists and biosynthesis inhibitors [Bazil et al., 1992; Clozel et al., 1993; Nishikibe et al., 1993; Tamirisa et al., 1995]. The inhibitor of ECE, phosphoramidon reduces the MABP in the spontaneously hypertensive rat (SHR) [McMahon et al., 1991]. In a similar model the endothelin ET_A antagonist BQ-123 reduces the MABP in the stroke prone SHR while no alterations are observed in the SHR [Nishikibe et al., 1993]. The combined endothelin ET_A/ET_B receptor antagonists have been demonstrated to reduce the MABP in some SHR and DOCA-salt SHR [Clozel et al., 1993].

Elevations in plasma ET-1 levels in patients suffering from pulmonary hypertension have been detected [Stewart et al., 1991]. The lung is the main organ involved in the clearance of ET-1 from the systemic circulation (approximately 80% of circulating ET-1 cleared from the lung) and there are suggestions that the aetiology of pulmonary hypertension may include the impaired clearance of ET-1 and resulting in pulmonary vasoconstriction. Increased expression of ET-1 mRNA in experimental models and in patients with pulmonary hypertension have been measured [Shirakami et al., 1991; Elton et al., 1992; Chang et al., 1993]. The hypoxia induced stimulation of ET-1 release in the lung and increased sensitivity of pulmonary arteries and arterioles to the contractile actions of ET-1 have implicated the endothelins in the development of pulmonary hypertension [Shirakami et al., 1991].

A role for the endothelins in the control of renal blood flow, glomerular filtration rate and Na+/water transport has been suggested [Cao & Banks, 1990; Chou et al., 1990; Perico et al., 1991]. Expression of pre pro ET-1 mRNA has been demonstrated in bovine glomerular endothelial cells and mRNA expression could be enhanced by bradykinin, thrombin, ATP and platelet aggregating factor (PAF) [Lopez-Farre et al., 1991]. Glomerular epithelial cell cultures release endothelin-1 and ET-1 binding sites have been identified in the glomeruli and medulla [Kohzuki et al., 1989; Kloog & Sokolovsky, 1989]. The systemic infusion of ET-1 in the renal cortex and medulla are mediated by ET_B receptors [Pollock & Opgenorth, 1993]. Alongside the reductions in renal blood flow, intravenous administration of ET-1 also reduces the glomerular filtration rate and sodium excretion [Cao & Banks, 1990; Perico et al., 1991]. ET-1 stimulates mitogenesis in mesangial cells and stimulates the production of PDGF and the immediate early genes (Fos, Jun) [Jaffer et al., 1990; Simonson et al., 1992]. Increases in ET-1 binding and receptor numbers have been reported following renal ischaemia and reperfusion [Clozel et al., 1991; Nambi et al., 1993]. Interventions with ET antibody or receptor antagonist such as BQ-123, SB209670 and bosentan prevent renal failure and damage to renal tubules following renal ischaemia [Mino et al., 1992; Clozel et al., 1993; Douglas et al., 1995].

Tracheal epithelial cell cultures also secrete ET-1. The production of ET-1 in these cultures can be regulated by TGF-ß, cytokines, interleukins and thrombin [Endo et al., 1992]. ET-1 is a potent constrictor of tracheal and bronchial smooth muscle *in vivo* and *in vitro* [Rubanyi & Polokoff, 1994] and a role for the endothelins in the mediation of respiratory disorders has been suggested [Gandhi et al., 1994; Rubanyi & Polokoff , 1994]. The contractions of the tracheal and bronchial smooth muscle are potentiated following the removal of the endothelium [Cardell et al., 1993; Hay et al., 1993]. These observations indicated that relaxant mediators such as PGI_2 may be released and attenuate the contractile effects of endothelin [Satoh et al., 1992].

In the gastro-intestinal tract, pre pro ET-1 mRNA has been identified in myenteric neuronal cells [Ota et al., 1991]. Gastric epithelial cells produce ET-1. ET-1 is a potent constrictor of stomach smooth muscle *in vivo* and *in vitro* [Ota et al., 1991]. The contractions are thought to be mediated by the endothelin ET_A receptors, however, in the guinea pig ileum ET-1 elicits a biphasic response [Lin & Lee, 1992]. The efficacy of endothelin ET_B receptor antagonists has suggested that the dilatation may be mediated by endothelin ET_B receptors [Lin & Lee, 1992; Hiley et al., 1992]. In longitudinal sections of the ileum, endothelin ET_B receptor antagonists attenuate the ET-1 mediated contractions [Urade et al., 1992; Yoshinaga et al., 1992].

There has been speculation on the role of the endothelins in the development of cerebrovascular disease. Experimental and clinical data from a number of investigators have demonstrated increases in plasma and cerebrospinal fluid (CSF) endothelin immunoreactivity following subarachnoid haemorrhage and ischaemic stroke [Ziv et al., 1992; Gang-zhi et al., 1993; Barone et al., 1994]. Elevations in endothelin immunoreactivity in brain tissue and CSF has been demonstrated in experimental models focal and global cerebral ischaemia [Barone et al., 1994; Giuffrida et al., 1992; Yamashita et al., 1994; Willette et al., 1992]. There is some controversy regarding the temporal profile for these increases in cerebral ischaemia since some investigators have demonstrated increases in the acute phase following ischaemia (2-3 hr) while others have reported increases at chronic time points (>24 hours) [Barone et al., 1994; Duverger et al., 1992]. The significance of the alteration

in endothelin levels at acute and chronic time points is at present unclear but has important implications for the administration of endothelin receptor antagonists following a cerebral ischaemic episode.

Exogenously applied endothelin-1 does not demonstrate neurotoxic potential when applied to cortical neuronal cell cultures and has no effect on the markers of neuronal cell viability [Lustig et al., 1992; Nikolov et al., 1993]. The administration of exogenous endothelin-1 into the cerebroventricular system exacerbated the neuronal damage associated with focal cerebral ischaemia in the mouse [Nikolov et al., 1993]. The intracisternal administration of exogenous endothelin-1 in rats, results in brainstem ischaemia [Macrae et al., 1991]. The topical administration of endothelin-1 onto a major cerebral artery, e.g. the middle cerebral artery, induces marked reductions in CBF in the cortex and striatum and ischaemic neuronal damage in these areas [Fuxe et al.., 1992; Robinson et al., 1990; Macrae et al., 1993; Sharkey et al., 1993].

Aims of Thesis

Since the primary publication describing the structure and functions of the endothelins, there have been rapid developments describing the role of the endothelins in physiology and pathophysiology and the therapeutic potential of the endothelin receptor antagonists. The primary aim of this thesis was to examine the physiologic and pathophysiologic role of the endothelins in the cerebral circulation by using endothelin receptor antagonists (notably bosentan) whose cerebrovascular pharmacology has been described in this thesis.

In the peripheral vasculature the constrictor and dilator effects of the endothelins are mediated variously by endothelin ET_A and endothelin ET_B receptors. In contrast, there is a paucity of information regarding the endothelin receptors and their anatomical location in cerebral arteries and arterioles. In the present thesis the endothelin receptor subtypes in cerebral arteries and arterioles were examined. These investigations utilised functional *in vitro* and *in vivo* techniques in cerebral blood vessels. The functional *in vitro* investigations utilised the rabbit basilar artery. The identification of receptor subtypes was performed using ET-1, ET-3, the endothelin ET_A selective receptor antagonist BQ-123, the combined endothelin ET_A/ET_B receptor antagonist Bosentan and the endothelin ET_B receptor agonist BQ-3020. The *in situ* characterisation of endothelin receptor subtypes was performed in feline cerebral resistance arterioles. BQ-123 was used as an antagonist for endothelin ET_A receptors while BQ-3020 was used as an endothelin ET_B receptor agonist.

The cerebrovasculature (particularly the vessels which contribute to cerebrovascular resistance) is minimally affected by intravascular agents which poorly penetrate the blood-brain barrier. It has been argued that endothelin-1 and endothelin receptor antagonists must gain access to the adventitial surface of cerebral blood vessels (e.g. intraventricular or topical administration) to produce direct vasomotor effects in vivo in the cerebral circulation. The investigation were designed to evaluate the efficacy of the non-peptide combined endothelin ET_A/ET_B receptor antagonist Bosentan and its ability to penetrate the blood-brain barrier. The investigations were carried out in cerebral resistance arterioles using the cranial window preparation in the

cat. The efficacy of Bosentan was assessed by the perivascular co-application of Bosentan with exogenous ET-1. The blood-brain barrier permeability was assessed by the ability of the intravenous administration of Bosentan to attenuate the vasoconstriction induced by the perivascular application of exogenous ET-1. The cerebrovascular effects of Bosentan were contrasted with the effects of a non-peptide endothelin ET_A receptor antagonist PD155080 and the peptide endothelin ET_A/ET_B receptor antagonist PD145065.

Recent evidence has indicated a pathophysiologic role for the endothelium in cerebrovascular diseases. The availability of non-peptide endothelin receptor antagonists that penetrate the blood-brain barrier has facilitated investigations into the cerebrovascular role of the endothelins. The investigations in this thesis examined the potential role of endothelins in regulating cerebral blood flow following cerebral ischaemic injury.

Transient global cerebral ischaemia in the rat often results in a period of post-ischaemic hypoperfusion. The aetiology of post-ischaemic hypoperfusion is unclear but vasoconstriction of the cerebral arterioles has been suggested. The ability of Bosentan to alter post-ischaemic cerebral hypoperfusion following transient global cerebral ischaemia was examined using two severities of ischaemia and two techniques to measure CBF ((^{14}C)-iodoantipyrine autoradiography and hydrogen clearance). Severe global cerebral ischaemia was induced by the bilateral occlusion of the common carotid arteries and the induction of haemorrhagic hypotension for 15 min. Moderate global cerebral ischaemia was induced by the bilateral occlusion of the common carotid arteries for 15 min.

Brain injury involving haemorrhage, e.g. subarachnoid haemorrhage and subdural haematoma, is often associated with marked reductions in cerebral blood flow and ischaemic neuronal damage. The mediators of the reductions in cerebral blood flow and neuronal damage are unclear but the release of vasoactive substances from the blood has been speculated. The ability of Bosentan to alter CBF following subdural haematoma was also examined. CBF was measured using (^{14}C)-iodoantipyrine autoradiography and at 2.5 hours following the induction of the

subdural haematoma. Despite the existence of multiple homeostatic mechanism to protect blood flow to brain, constrictor mechanisms which limit tissue mechanisms which limit tissue perfusion to the ischaemic and peri-ischaemic regions are well recognised in cerebral ischaemia. A variety of mechanisms including vasoconstriction, have been proposed to account for the submaximal dilatation in the ischaemic penumbra following focal cerebral ischaemia. The role of the endothelins in the regulation of post-ischaemic vascular diameter was examined following permanent occlusion of the MCA in the cat. The ability of Bosentan to alter the calibre of post-occlusion cerebral resistance arterioles was contrasted with the effects of the endothelin ET_A receptor antagonist PD155080 in the same preparation.

CHAPTER 2

MATERIALS AND METHODS

2.1 Rabbit Basilar Artery *in vitro*

Surgical Preparation

Adult male New Zealand white rabbits (weight 2.5-4 kg) were killed by an overdose of sodium pentobarbitone (Euthatal, 200 mg/ml) administered into the marginal ear vein. The brain was dissected out and placed in oxygenated ice cold Krebs' buffer (mM concentrations: NaCl 118; KCl 4.7; KH_2PO_4 1.2; $NaHCO_3$ 25; $MgSO_4$ 1.2; $CaCl_2$ 2.5; Glucose 11). Potassium (50 mM) substituted Krebs' was made by the direct substitution of NaCl with KCl The basilar artery was carefully freed of the surrounding arachnoid membrane using a dissecting microscope and 2-4 mm segments were mounted on to stainless steel hooks (diameter ~ 200μm) positioned through the lumen of the artery.

Experimental protocol

The vessel segments were placed in 10 ml siliconised organ baths. One hook was secured to the bottom of the bath and the other was secured to an isometric tension transducer and a resting tension of 500 mg applied. The preparations were suspended in Krebs' buffer at 37^oC continuously perfused with 95% O_2 / 5% CO_2 . Vessel segments were allowed to equilibrate for a period of 60 min during which time the fluid in the bath was replaced every 10-15 min.

After the equilibration period, the bathing medium was replaced with 50 mM K^+ substituted Krebs' buffer and the contraction due to potassium induced depolarisation was measured. The vessels were allowed to attain a stable baseline and acetylcholine (10 μM) was added to check for the integrity of the endothelium. The buffer was replaced with normal Krebs' buffer and a recovery period of 15 min was allowed. Previous investigations have indicated that concentration of acetylcholine used in the present studies would have elicited marked relaxant responses on the rabbit basilar artery [Rand & Garland, 1992; Plane & Garland, 1993]. However, no histological evidence for the de-endothelialisation of the basilar artery segments was obtained.

The vessel segments were incubated with vehicle (DMSO), BQ-123 (0.1 - 10 μM) or Bosentan (1 - 10 μM) for a period 30 min before cumulative concentration response curves to ET-1, ET-3 or BQ-3020 were constructed. Single cumulative concentration response curves were constructed on each segment. At the lower concentrations (0.1 nM - 1 nM) of ET-1, ET-3 and BQ-3020, an equilibration period of 2 - 3 min was permitted before the next concentration of agonist added [Trace A]. At the higher concentrations (>1 nM), the contractile responses were allowed to plateau before the next concentration of agonist was added [Trace A]. The plateau of the response was typically reached in 30-60 seconds.

Statistical Analysis

The mean EC_{30} values were analysed using a one way analysis of variance (ANOVA) and Student's *t* - test with Bonferroni correction factor for multiple group comparisons. The pA_2 values were calculated from the method described by Arunlakshana and Schild (1959). Briefly, dose ratios (DR) were calculated from the concentration of agonist (ET-1 or ET-3) required to produce a response that was 30% of the maximum. The dose ratios were calculated in the presence and absence of the endothelin receptor antagonists. The pA2 was calculated using the equation:

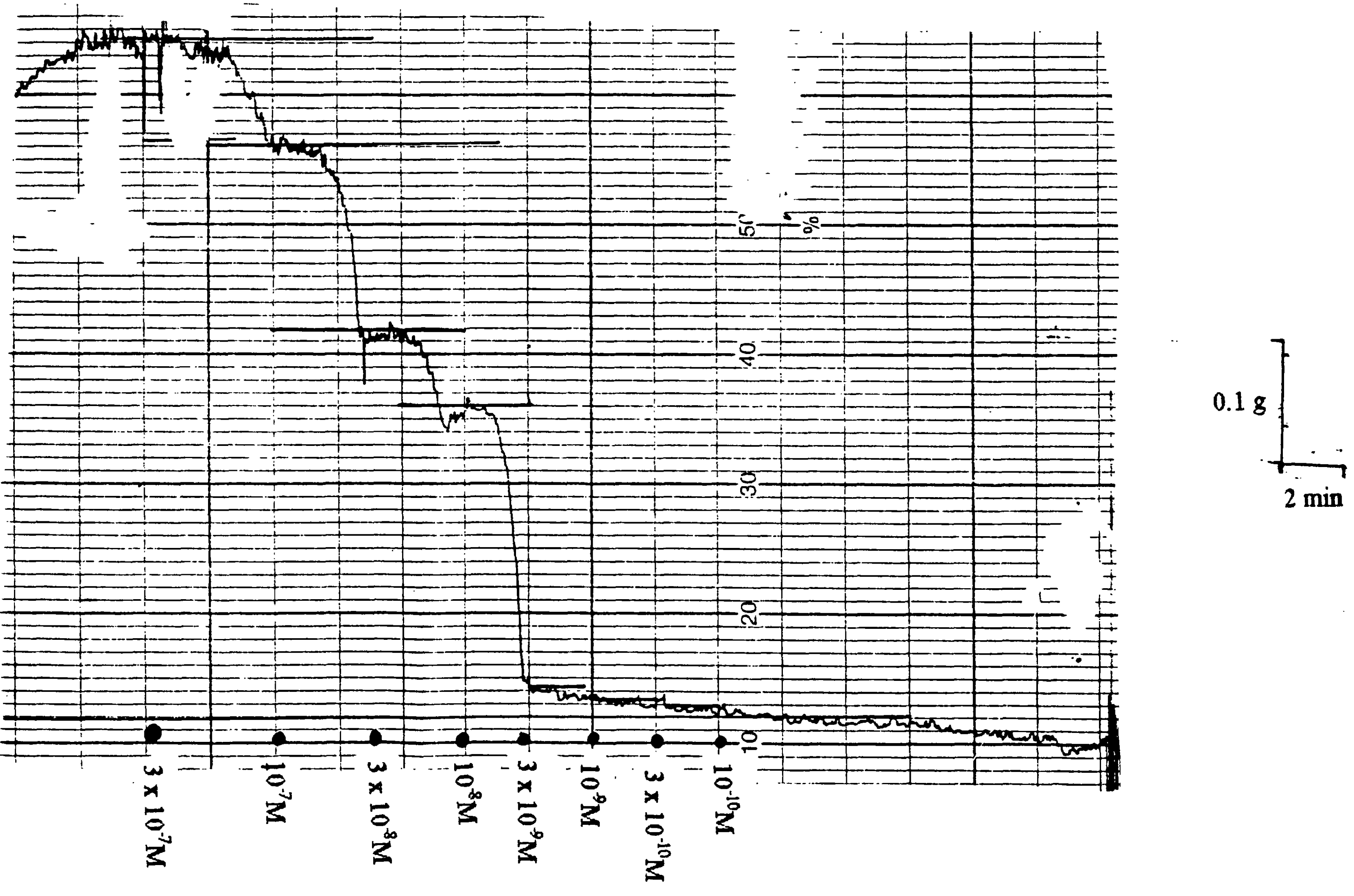
$$-\log(DR-1) + \log(\text{concentration of antagonist})$$

The pA_2 values at each concentration of antagonist used were averaged to give a mean pA_2.

Trace A

Rabbit Basilar Artery *in vitro*

Endothelin-1 concentration response curve



2.2 Measurement of calibre of feline pial arterioles *in situ*

Surgical Preparation

The experiments were performed on 22 adult cats of either sex weighing between 2-3 kg. Anaesthesia was induced using alphaxolone/alphadolone (Saffan, Glaxo) administered into the radial vein. The animal was then intubated and positive pressure ventilation with $N_20/0_2$ (70%/30%) initiated. The femoral arteries and veins were cannulated for the monitoring of arterial blood pressure and arterial blood status gas and for the administration of fluid and drugs respectively. Anaesthesia was maintained using α-chloralose (60 mg/kg) and further supplements of 1-2 ml of α-chloralose (1%) were administered to prevent the return of the corneal reflex.

Arterial blood samples were taken at regular intervals for monitoring of blood gases. The animals were maintained normocapnic by adjusting the stroke volume and metabolic acidosis was controlled by the administration of sodium bicarbonate (8.4%). Core temperature was measured with a rectal thermometer and the temperature maintained at 37°C by means of a heating blanket controlled by the rectal thermometer.

The animal was placed in a stereotactic frame. After a midline incision, the scalp was retracted and sutured onto a metal ring to form a well over the calvarium. The temporalis muscle was retracted and a rectangular craniectomy (2.5 cm x 1.5 cm) was made over the parietal cortex using a saline cooled dental drill. The exposed dura was bathed with mineral oil at 38°C. There was a continuous flow of oil at the rate of approximately 1 ml/min. With the aid of a stereomicroscope (Bausch & Lomb) the dura was excised and then reflected laterally. Bipolar diathermy was used to control bleeding from the dural vessels. The inspired gas mixture was then altered to 0_2 supplemented air (approximately 25% 0_2). In a separate group of animals the lingual artery was exposed and catheterised. The catheter was advanced into the carotid artery and subsequently used for the intravascular infusions of the ET_B receptor agonist BQ-3020.

Measurement of Arteriolar Calibre

Arteriolar calibre was measured by the method of Baez (1966) using an image splitter linked through a closed circuit video display system. The system was calibrated at x40 and x70 against threads of known diameter and this allowed for the direct measurement of vascular diameter in absolute units (μm). The individual pial vessels were viewed through a stereomicroscope and the arteriolar diameter was measured from the degree of shear applied to the image splitter in order to tangentially appose the two images. The shearing screw on the image splitter was connected to a chart recorder allowing a calculation of the calibre of pial arterioles. The image of the arteriole on the video monitor was split using the image shearing device and multiple measurements of the split image were taken [Trace B]. Following the measurements, substances were injected into the perivascular space, the split image realigned and multiple measurements of the realigned image were taken. The addition of vasoconstrictor agents such as ET-1 resulted in an increase in the gap between the split image, while the addition of vasodilator agents such as BQ-3020 resulted in an overlap of the split image. The vessel diameter was typically measured pre-insertion, pre-injection and post-injection and the vessel diameter was monitored over a period of 1-2 min. The diameter of the pial arterioles was measured at the peak of the alteration in the arteriolar calibre (typically between 30-45 seconds following the perivascular microinjection) [Trace B]. The peak of the vasoconstrictor response elicited by endothelin-1 was followed by a sustained reduction in arteriolar calibre (plateau phase) [Trace B]. The plateau phase may be indicative of the contractile response attaining an equilibrium.

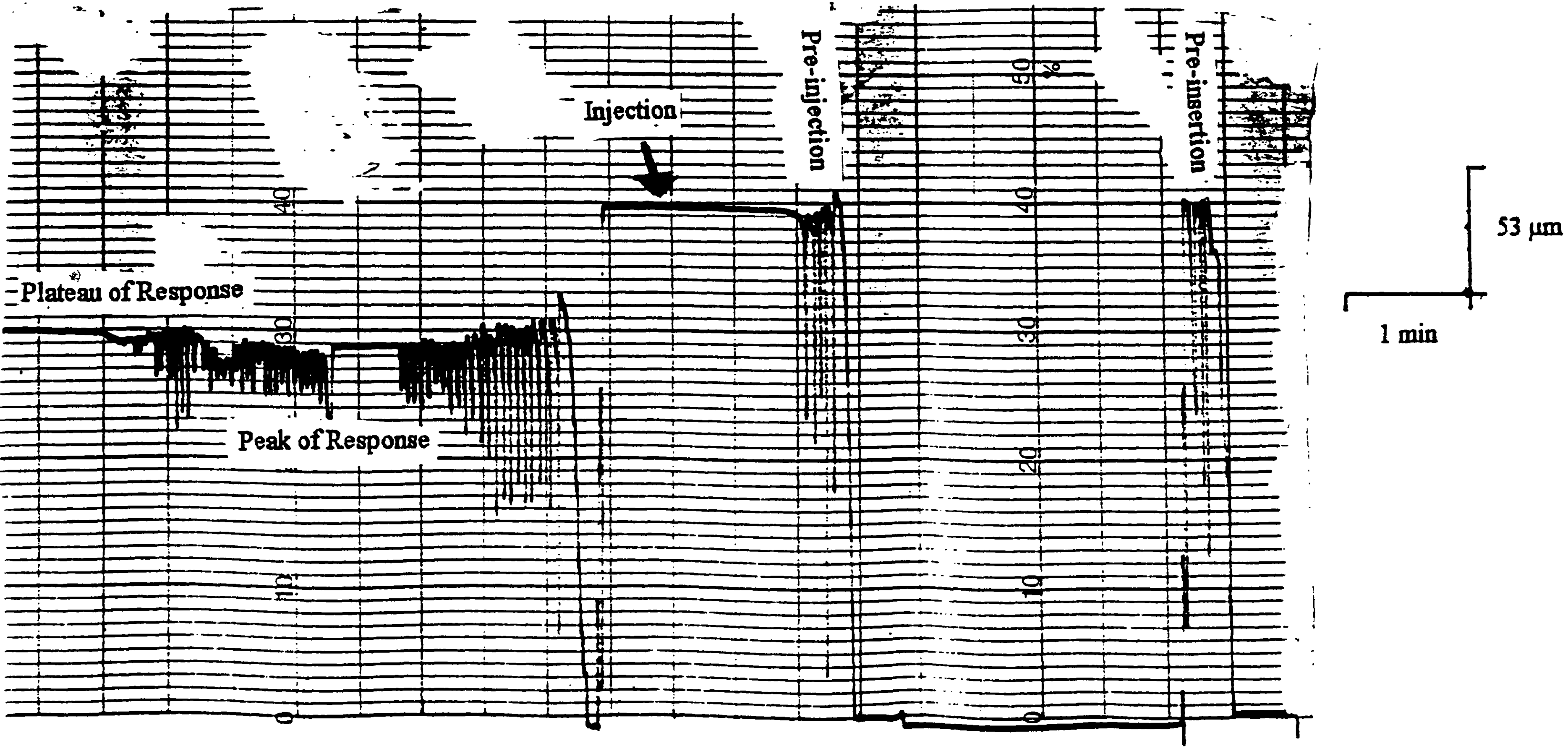
Administration of Drugs

Sharp glass micropipettes (tip diameter 10-12μm) were vacuum filled with either artificial cerebrospinal fluid (CSF), 10 mM K^+ CSF or drugs dissolved in CSF. The composition of the CSF was Na^+ 156 mM, K^+ 3 mM, Ca^{2+} 2.5 mM, HCO_3^- 12 mM, Cl^- 152 mM with the pH adjusted to 7.2 by aeration with 5% CO_2/95% O_2. Solutions containing K^+ 10 mM were prepared by equimolar substitution of KCl for

Trace B

Feline Pial Arterioles *in situ*

Endothelin-1 induced reduction in arteriolar calibre



NaCl. Alkalotic CSF (pH 7.45) were prepared by equimolar substitution of $Na^{+}HCO_3^{-}$ (22 mM) for NaCl. All solutions were prepared freshly for each study. The filled pipettes were stored under mineral oil until required. The substances were applied by subarachnoid microapplication using a micromanipulator to position the pipettes in the perivascular space around the vessel. Small volumes (5μl) were delivered by a pressure ejection system. Individual sites on the arterioles were studied on only a single occasion. Perivascular microapplication of endothelin-1 (10 nM) were employed as the challenge dose as this concentration is the EC_{50} value in this preparation (Robinson and McCulloch, 1990).

The effects of Bosentan (0.3 - 300 μM), PD155080 (0.3 - 30 μM), PD145065 (0.03 - 3 μM), BQ-123 (0.1 - 10 μM), BQ3020 (0.001 - 1 μM) and autologous blood were examined following their perivascular microapplication. Bosentan (17 μmol/kg), the choline salt of PD155080 (19.2 μmol/kg) and PD145065 (30 μmol/kg) were dissolved in isotonic saline and administered as slow intravenous injections (5-7 min). The choline salt of PD155080 elicited transient alterations in mean arterial pressure during the intravenous injection and resulted in pauses in the drug delivery until they subsided. The investigations were repeated using choline chloride dissolved in isotonic saline administered intravenously as a vehicle control for the PD155080 investigations. Perivascular microapplications of endothelin-1 (10 nM) and alkalotic CSF (7.45) were made 15-75 min after vehicle, Bosentan, PD155080 or PD145065 administration. The effect of intravenous Bosentan on the constrictions elicited by the microapplication of blood was examined. In the studies examining the effects of PD145065, an intravenous bolus dose of endothelin-1 (1 nmol/kg) was administered 120 min after the initial administration of PD145065.

The ability of Bosentan and PD155080 to reverse an established ET-1 induced vasoconstriction was also examined. The calibre of the arteriole was recorded for 5 - 15 minutes following the perivascular microapplication of ET-1 (10 nM). CSF, Bosentan (30 μM) or PD155080 (30 μM) were applied to the ET-1 constricted vessels between 5 and 15 minutes subsequent to application of ET-1. The vessel diameter was measured pre-insertion of micropipettes, pre- and post- injection of substances and the

vessel diameter was monitored over a period of 1 - 2 minutes. BQ-3020 for intravascular infusions was dissolved in 0.9% saline and infused at a rate of 0.15 ml/min.

Statistical Analysis

The results were analysed using a one way analysis of variance followed by Student's *t*-test using a Bonferroni correction factor for multiple group comparisons. The ability of Bosentan or PD155080 to reverse ET-1 induced vasoconstriction was assessed by unpaired Student's *t*- test. Data are expressed as mean ± S.E.M. of the percent change in pial arteriolar calibre from pre-injection baseline; n = the number of arterioles examined.

2.3 Cerebral Blood Flow following Bilateral Common Carotid Artery Occlusion with Concomitant Haemorrhagic Hypotension in the Rat

Surgical Preparation

The experiments were carried out in 29 adult male Sprague Dawley rats (305 - 439 g). The animals were initially anaesthetised with 4% halothane, tracheotomised and mechanically ventilated with a gas mixture of 70% N_2O / 30% O_2 containing halothane (0.75 - 1.5%). Both femoral arteries and veins were catheterised for the monitoring of arterial blood pressure, blood gas status and for the administration of drugs and fluids. The common carotid arteries were isolated from surrounding tissue by blunt dissection and loose loop ligatures (2-0 silk suture) placed around the arteries.

Hydrogen electrode placement

The animals were positioned in a stereotactic frame (Kopf instruments) and, following a midline incision, the surface of the skull exposed . Two burr holes were drilled to expose the surface of the dura. The dura was incised and teflon coated platinum electrodes (250 µm outer diameter, 1 mm tip exposed, Clark Electromedical) positioned in the left caudate nucleus (co-ordinates Anterior 1 mm; Medial 3 mm; Dorso-Ventral 4 mm) and in the right parietal cortex (Posterior 2.4 mm; Medial 4 mm; Dorso-Ventral 1 mm). The co-ordinates were taken (relative to bregma) from the atlas of the rat brain of Paxinos and Watson (1986). The burr holes were sealed with cyanoacrylate glue and the platinum electrodes cemented in place using dental cement. A silver reference electrode was placed subcutaneously on the back of the animals.

Experimental Protocol

Vehicle (0.9% saline) or Bosentan (17 µmol/kg) was administered as an intravenous bolus 10 min prior to the induction of ischaemia. The intravenous injections were carried out over a period of 3 minutes. The common carotid arteries were occluded and mean arterial blood pressure (MABP) reduced to between 40 - 50

mmHg by concomitant arterial haemorrhage for 15 min (Smith et al., 1984). The haemorrhaged blood was stored in a water bath at 37°C. Cerebral blood flow was restored by the removal of the carotid ligatures and the re-infusion of the blood via the femoral vein.

CBF measured by Hydrogen Clearance

Following an equilibration period of approximately 30 min, the platinum electrodes were connected to a reference circuit and polarised to +700 mV. A further period of 30 min was allowed for stabilisation following polarisation. Prior to the measurement of cerebral blood flow (CBF), the inspired gas mixture was altered to 45% O_2, 35% H_2 and 20% N_2O with 0.75% - 1.5% halothane. A saturation period of 10 min was allowed followed by 10 min for clearance. The CBF data were collected over a period of 1 min., 1 min. after the H_2 mixture had been switched off (to reduce artefacts due to recirculation of hydrogen). The data were fitted to an experimental curve and blood flow calculated using the initial slope index technique [Doyle et al., 1975; Young, 1980]. Measurements of cerebral blood flow were carried out prior to occlusion, 7.5 min after the occlusion of the carotid arteries, 5 min following the release of the carotid ligatures and at 30 min intervals for 3 hours during the reperfusion phase.

CBF measured by [^{14}C]- Iodoantipyrine Autoradiography

At 90 min following the release of the carotid ligatures, cerebral blood flow was measured using [^{14}C]- Iodoantipyrine as the radiotracer. The method utilised was modified from the method of Sakurada et al., 1978. Briefly, 50 µCi of radioisotope in 1.5 ml saline was administered as a ramped intravenous infusion over a period of 30 seconds. The infusion rate during the first 5 seconds was 50% of the final infusion rate. During the infusion 15 - 18 samples of arterial blood were collected on pre-weighed filter discs. At the end of the infusion of isotope, the animals were decapitated and the brain removed and frozen in isopentane at -42°C. The filter discs

Operational Equation for the Calculation of Cerebral Blood Flow using [^{14}C]- Iodoantipyrine

$$Ci\,(T) = \lambda K \int_0^T C_a \; e^{-K\,(T-t)}\; dt$$

Ci (T) = tissue concentration of tracer at given time (T) after intravenous injection of tracer

λ = tissue blood partition coefficient

Ca = tracer concentration in arterial blood

t = variable time

K = constant defined as $\frac{mF}{W\lambda}$

where m =1 (diffusion of tracer across blood-brain barrier)
F/W = rate of blood flow per unit mass of tissue

The rate of cerebral blood flow per unit mass (F/W) can be calculated from the final concentration of the ^{14}C in cerebral tissue (Ci (T) obtained from autoradiograms), the concentration of [^{14}C] - iodoantipyrine in arterial blood from time 0 to time T and the blood partition coefficient of [^{14}C]- iodoantipyrine (0.79)

(Sakurada et al., 1978)

were re-weighed and a 30% solution of hydrogen peroxide added to bleach the blood from the discs. [^{14}C] radioactivity was determined by liquid scintillation counting.

Twenty micron sections of the frozen brains were cut serially in a cryostat at -20°C and at 140 μm intervals, sections were collected onto glass coverslips and rapidly dried at 60°C. The coverslips were mounted onto cards and apposed to Kodak SB-5 film for 4 - 6 days. Pre-calibrated [^{14}C] standards (concentration range 44 - 2500 nCi/g tissue equivalents) were placed in the film cassettes. The autoradiograms were analysed using a computer based image analysis system (M4 MCID, Imaging Research Inc.). Six bilateral readings were taken from sections where the structure could be identified from the rat brain atlas of Paxinos and Watson (1986).

The values of cerebral blood flow were determined from the equation of Sakurada et al., 1978, with the knowledge of arterial blood history of [^{14}C], tissue levels of [^{14}C] and the tissue blood partition coefficient (0.79).

Statistical Analysis

The data for the two treatment groups, Bosentan and vehicle, were analysed using a Student's unpaired two tailed *t*-test. The data are expressed as mean ± S.E.M. of the percent change of CBF from pre occlusion baseline for the hydrogen clearance investigations and expressed as mean ± S.E.M. for the [^{14}C]-Iodoantipyrine CBF investigations (ml/100g/min).

2.4 Cerebral Blood Flow following Bilateral Common Carotid Occlusion in the Rat

Surgical Preparation

The experiments were carried out in 30 adult male Sprague Dawley rats (303 - 432 g). Surgical preparation was carried out as in 2.3.

Hydrogen electrode placement

Hydrogen electrode placement was carried out as in 2.3

Experimental Protocol

Vehicle (0.9% saline) or Bosentan (17 μmol/kg) was administered as an intravenous bolus 10 min prior to the induction of ischaemia. The intravenous injections were carried out over a period of 3 minutes. Unlike the protocol described in section 2.3, in these experiments the common carotid arteries were occluded for 15 min **without** the induction of systemic haemorrhagic hypotension. Cerebral blood flow was restored by the removal of the carotid ligatures. Plasma glucose samples were taken prior to occlusion and at 30 min into the reperfusion phase. At each flow determination a blood sample was taken to measure arterial blood gas status .

CBF measured by hydrogen clearance

CBF measured by hydrogen clearance was carried out as in 2.3

CBF measured by [^{14}C]- Iodoantipyrine Autoradiography

CBF measured by [^{14}C]- Iodoantipyrine Autoradiography was carried out as in 2.3

Statistical Analysis

The data for the two treatment groups, Bosentan and vehicle, were analysed using a Student's unpaired two tailed *t*-test. The data are expressed as mean ± S.E.M. of the percent change of CBF from pre occlusion baseline for the hydrogen clearance investigations and expressed as mean ± S.E.M.. for the [^{14}C]-Iodoantipyrine CBF investigations (ml/100g/min).

2.5 Cerebral Blood Flow following Subdural Haematoma in the Rat

Surgical Preparation

The experiments were carried out in 12 adult male Sprague Dawley rats (309 - 423 g). The animals were initially anaesthetised with 4% halothane, tracheotomised and mechanically ventilated with a gas mixture of 70% N_2O / 30% O_2 containing halothane (0.75 - 1.5%). Both femoral arteries and veins were catheterised for the monitoring of arterial blood pressure, blood gas status and for the administration of drugs and fluids.

The animals were positioned in a stereotactic frame (Kopf instruments) and, following a midline incision, the surface of the skull exposed. A burr hole (2 mm diameter) was drilled over the parietal cortex (co-ordinates Anterior 1 mm; Medial 3 mm relative to bregma [Paxinos & Watson, 1986]) and the dura excised. A blunt 25G J-shaped needle was positioned under the dura, the burr hole sealed with cyanoacrylate glue and cemented in place using dental cement.

Experimental Protocol

Vehicle (distilled water) or Bosentan (17 μmol/kg) was administered as an intravenous bolus 10 min prior to the induction of the haematoma. The intravenous injections were carried out over 3 minutes. Venous blood (400μl) was withdrawn from the femoral catheter and the haematoma induced by the infusion of the blood, via the subdural needle, over a period of 7 minutes.

CBF measured by [^{14}C]- Iodoantipyrine Autoradiography

At 150 min following the induction of the haematoma, cerebral blood flow was measured using [^{14}C]- Iodoantipyrine as the radiotracer. The method utilised was modified from the method of Sakurada et al. (1978) and as described in section 2.3.

Twenty micron sections of the frozen brains were cut serially in a cryostat at -20°C and at 140 μm intervals, sections were collected onto glass coverslips and rapidly dried at 60°C. The coverslips were mounted onto cards and apposed to Kodak

BMR-2 film for 3 days. Pre calibrated [^{14}C] standards (concentration range 44 - 2500 nCi/g tissue equivalents) were placed in the film cassettes. The autoradiograms were analysed by two approaches using a computer based image analysis system (M4 MCID, Imaging Research Inc.). In the first approach 6 bilateral readings were taken from sections where the structure could be identified from the rat brain atlas of Paxinos and Watson (1986).

The second approach determined volume of cerebral tissue in both hemispheres perfused with various levels of CBF using cumulative frequency distribution analysis. The image analyser was set for optical density thresholds corresponding to pre-determined increments of cerebral blood flow, i.e., 0 - 10, 10 - 20, 20 - 30, 30 -40, 40 - 50, 50 - 60, 60 - 70, 70 - 80, 80 - 90, 90 - 100, 100 - 150, >150 ml/100g/min. The distribution of cerebral blood flow in both hemispheres was assessed at the 8 coronal planes used for volumetric quantification of ischaemic damage, stereotactic co-ordinates (relative to bregma) anterior 10.5 mm, anterior 8.92 mm, anterior 7.19 mm, anterior 6.06 mm, anterior 5.15 mm, anterior 3.75 mm, anterior 2.18 mm, anterior 1.02 [Osborne et al., 1987]. The area of each hemisphere (on 3 sections) was delineated on the image analyser and distribution of CBF and area recorded. The area of tissue corresponding to each of the levels of blood flow was calculated and the volume of tissue at these levels of blood flow was determined by the integration of the areas at 8 coronal planes [Osborne et al., 1987].
Brain swelling in the ipsilateral hemisphere was calculated by subtracting the volume of the ipsilateral hemisphere from the volume of the contralateral hemisphere.

The values of cerebral blood flow were determined from the equation of Sakurada et al. (1978) with the knowledge of arterial blood history of [^{14}C], tissue levels of [^{14}C] and the tissue blood partition coefficient (0.79).

Statistical Analysis

The data from frequency distribution of cerebral blood flow, the cerebral blood flow in the individual structures and the brain swelling were analysed using an unpaired Student's *t*-test. The differences in the cerebral blood flow between structures

in the ipsilateral hemisphere and contralateral hemisphere were analysed using a paired Student's *t*-test.

2.6 Measurement of Pial Arteriolar Calibre following Permanent MCA Occlusion in the Cat

Surgical preparation

The experiments were performed on 7 adult cats of either sex weighing between 2-3 kg. Anaesthesia was induced using alphaxolone/alphadolone (Saffan, Glaxo) administered into the radial vein. The animal was then intubated and positive pressure ventilation with $N_20/0_2$ (70%/30%) initiated. The femoral arteries and veins were cannulated for the monitoring of arterial blood pressure and arterial blood status gas and for the administration of fluid and drugs respectively. Anaesthesia was maintained using α-chloralose (60 mg/kg) and further supplements of 1-2 ml of α-chloralose (1%) were administered to prevent the return of the corneal reflex.

Arterial blood samples were taken at regular intervals for monitoring of blood gases. The animals were maintained normocapnic by adjusting the stroke volume and metabolic acidosis was controlled by the administration of sodium bicarbonate (8.4%). Core temperature was measured with rectal thermometer and the temperature maintained at 37°C by means of a heating blanket controlled by the rectal thermometer.

The animal was placed in a stereotactic frame. After a midline scalp incision, the scalp was retracted and sutured onto a metal ring to form a well over the calvarium. The temporalis muscle was retracted and a rectangular craniectomy (2.5 cm x 1.5 cm) was made over the parietal cortex using a saline cooled dental drill. The exposed dura was bathed with mineral oil at 38°C. There was a continuous flow of oil at the rate of approximately 1 ml/min. With the aid of a stereomicroscope (Bausch & Lomb) the dura was excised and then reflected laterally. Bipolar diathermy was used to control bleeding from the dural vessels. The inspired gas mixture was then altered to 0_2 supplemented air (approximately 25% 0_2).

The left middle cerebral artery (MCA) was exposed and occluded via a transorbital approach by a neurosurgeon (Mr. Galbraith). Briefly, with microsurgical

techniques, the contents of the left orbit were exenterated and the optic foramen enlarged with a dental drill to expose the dura mater overlying the MCA close to its origin. Under the operating microscope the dura was incised and the MCA exposed. The trunk of the MCA, all collateral vessels and all visible branches of the lenticulostriate arteries were coagulated with bipolar diathermy and sectioned with microscissors.

Measurement of Arteriolar Calibre

Arteriolar calibre was measured by the method of Baez (1966) using an image splitter linked through a closed circuit video display system. The system was calibrated at x40 and x70 against threads of known diameter and this allows for the direct measurement of vascular diameter in absolute units (μm). The individual pial vessels were viewed through a stereomicroscope and the arteriolar diameter was measured from the degree of shear applied to the image splitter in order to tangentially appose the two images. No perivascular microinjections were made during the first 30 min after MCA occlusion. During this period, the vasomotor responses of 25 pre-selected vessels were monitored intermittently and their calibre after MCA occlusion contrasted with their calibre immediately prior to occlusion. CSF, Bosentan (30 μM) or PD155080 (30 μM) were applied to arterioles in the period 30 - 210 min following MCA occlusion. The vessel diameter was typically measured pre-insertion, pre-injection and post-injection and the vessel diameter was monitored over a period of 1-2 min. A detailed description of the surgical preparation and measurement technique has appeared previously (Robinson and McCulloch, 1990).

Administration of Drugs

Sharp glass micropipettes (tip diameter 10-12 μm) were vacuum filled with either artificial cerebrospinal fluid (CSF), 10 mM K^+ CSF or drugs dissolved in CSF. The composition of the CSF was Na^+ 156 mM, K^+ 3 mM, Ca^{2+} 2.5 mM, HCO_3^- 12 mM, Cl^- 152 mM with the pH adjusted to 7.2 by aeration with 5% CO_2/95% 0_2. All solutions were prepared freshly for each study. CSF, Bosentan (30μM) or PD155080

(30μM) were applied to arterioles in the period 30 - 210 min following MCA occlusion. The substances were applied by subarachnoid microapplication using a micromanipulator to position the pipettes in the perivascular space around the vessel. Small volumes (5 μl) were delivered by a pressure ejection system. Individual sites on the arterioles were studied on only a single occasion.

Statistical Analysis

The data from the vessels overlying the parasagittal gyrus and the dilated and constricted vessels overlying the suprasylvian and ectosylvian gyri were analysed (Bosentan or PD155080 versus CSF) using an unpaired Student's *t*-test. A two way analysis of variance with time as a co-variant was used to analyse the temporal component of the effects of Bosentan and CSF on post-ischaemic dilated vessels. All results are expressed as mean ± S.E.M.. of the percent change of pial arteriolar calibre from pre-injection baseline; n = the number of arterioles examined.

CHAPTER 3

RESULTS

3.1 ENDOTHELIN RECEPTORS IN THE CEREBROVASCULATURE

3.1.1 Rabbit Basilar Artery *in vitro*

All the segments utilised in the study elicited a contraction when exposed to 50 mM Potassium substituted Krebs' (50 mM K^+). The mean response was 348 ± 21 mg (n = 42). The application of acetylcholine (10 μM) resulted in a small relaxation of the 50 mM K^+ response (<10%) indicating the absence of any significant endothelium dependent relaxation. Previous investigations have indicated that concentration of acetylcholine used in the present studies would have elicited marked relaxant responses on the rabbit basilar artery [Rand & Garland, 1992; Plane & Garland, 1993]. However, no histological evidence for the de-endothelialisation of the basilar artery segments was obtained.

The addition of endothelin-1 (at a concentration greater than 1 nM) to the organ baths resulted in a contraction of the basilar artery segments. The contractile responses reached a plateau, at each concentration, in approximately 30-45 seconds. However, a maximal contraction was not achieved with a concentration of 10^{-7} M of endothelin-1.

Bosentan

The addition of Bosentan at a concentration of 1 or 10 μM did not induce any contractile or relaxant effects on rabbit basilar artery segments. At a concentration of 1 μM, Bosentan did not appear to have any effect on the dose response curve to ET-1. However, 10 μM Bosentan elicited a rightward shift in the dose response curve that is consistent with the feature of competitive antagonism. However, the rightward shift was also accompanied by what appeared to be a steepening of the dose response curve [Figure 7]. The estimated pA_2 value for Bosentan in this preparation was 5.1. There were no significant differences in the mean EC_{50} values [Table 4].

BQ-123

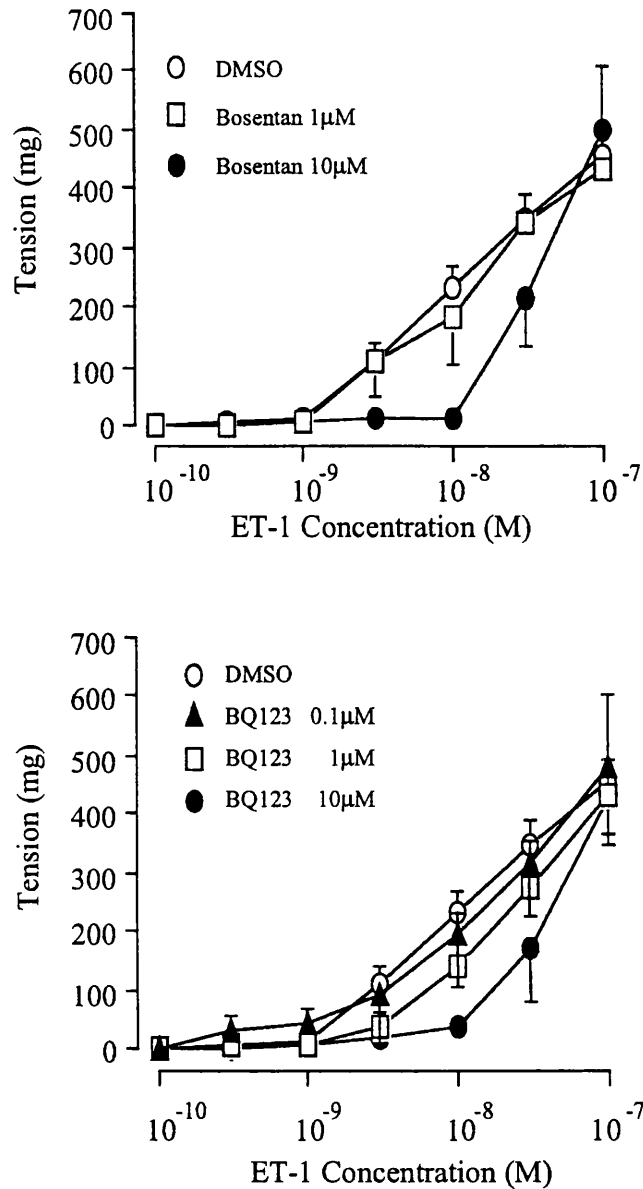


Figure 7 Concentration response curves demonstrating the antagonism of ET-1 induced vasoconstriction by bosentan (top) and BQ-123 (bottom) in the rabbit basilar artery. Data are expressed as mean ± S.E.M (n = 2 - 12 in each group). The pA_2 values obtained were 5.1 for bosentan and 5.3 for BQ-123.

Table 4

Potency of endothelin receptor agonists and antagonists in the rabbit basilar artery *in vitro*

	EC_{30} (nM)	95% C.I. (nM)	n (number of segments examined)
ET-1	11.54	0.3 - 23.4	11
ET-1 + Bosentan 1 µM	8.17	4.5 - 11.9	3
ET-1 + Bosentan 10 µM	15.37	9.9 - 20.9	5
ET-1 + BQ-123 0.1 µM	14.84	0.12 - 29.6	2
ET-1 + BQ-123 1 µM	22.74	6.72 - 38.6	4
ET-1 + BQ-123 10 µM	24.66	12.92 - 36.4	5
ET-3	194	102 - 287	4
ET-3 + BQ-123 1 µM	2420 * †	1700 - 3100	4

* $P < 0.01$ significant difference relative to ET-3
† $P < 0.01$ significant difference relative to ET-1 + BQ-123 1 µM

EC_{30} - concentration of agonist producing 30% of maximal response

C.I.- confidence limits

The addition of BQ-123 at the concentrations (0.1 - 10 μM) did not elicit any discernible contractile or relaxant effects on rabbit basilar artery segments. At concentrations of 1 μM and 10 μM BQ-123 elicited a rightward shift in dose response curve to endothelin suggesting competitive antagonism of ET-1 induced contractile effect [Figure 7]. This gave an estimated pA_2 value of 5.3. There was no significant difference in the mean EC_{30} values [Table 4].

ET-3

The addition of ET-3 elicited marked contractions of the rabbit basilar artery segments. ET-3 was approximately 100 fold less potent than ET-1 in eliciting a contractile response in the rabbit basilar artery. BQ-123 (1 μM) effected a rightward shift of the ET-3 concentration response curve indicating competitive antagonism of endothelin ET_A receptors. The pA_2 value obtained was 7.2 which is approximately 100 fold greater than the pA_2 obtained for BQ-123 against ET-1 in the same tissue [Figure 8]. There was significant difference in the mean EC_{30} values between ET-3 and BQ-123 treated segments and the segments treated with BQ-123 and ET-1 or ET-3 [Table 4].

BQ3020

The addition of BQ3020 to the bath resulted in a small contraction of the basilar artery. The magnitude of this response was most marked at concentrations in excess of 0.1 μM, although the response was much smaller than the contractile responses elicited by ET-1 or ET-3 [Figure 8].

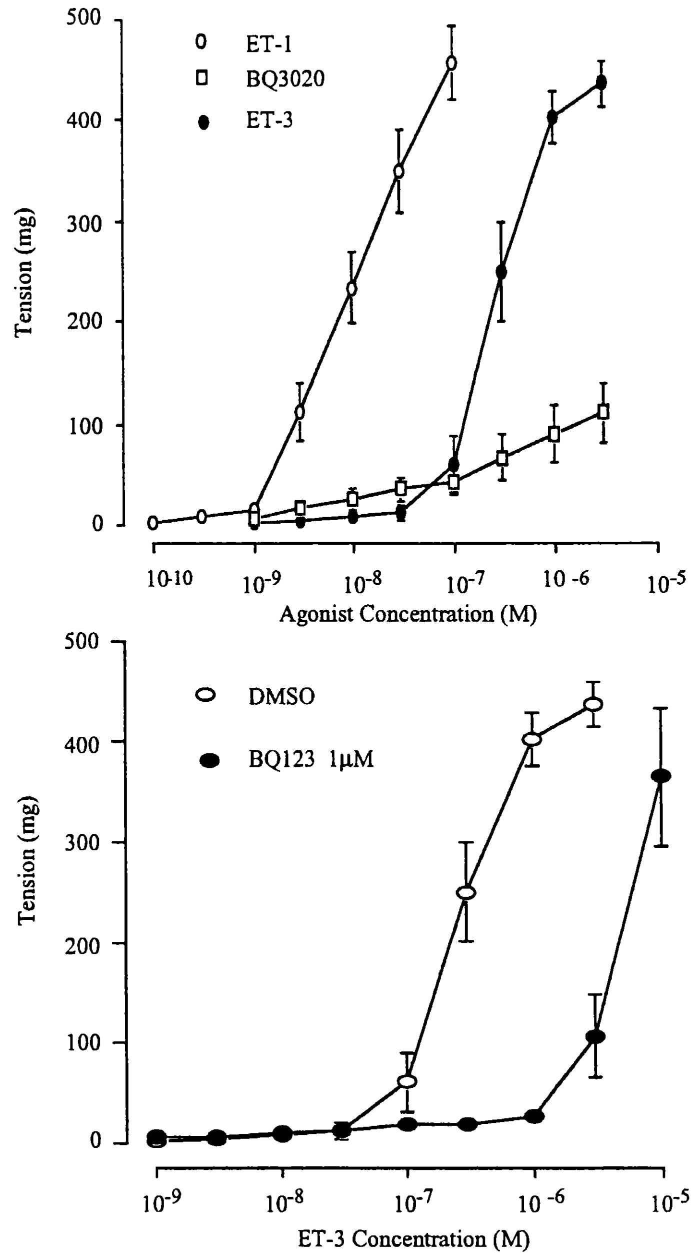


Figure 8 Concentration response curves demonstrating vasoconstriction induced by ET-1, ET-3 and BQ-3020 (top) and the antagonism of ET-3 induced vasoconstriction by BQ-123 (bottom) in the rabbit basilar artery. Data are expressed as mean ± S.E.M (n = 4 - 11 in each group). The pA_2 value obtained was 7.1 for BQ-123.

3.1.2 Feline Cerebral Arterioles *in situ*

At the outset of the study, mean arterial blood pressure (MABP) was 97 ± 3 mmHg, arterial carbon dioxide tension was 30 ± 1 mmHg and arterial oxygen tension was 201 ± 5 mmHg. During the course of the study, the physiological variables did not vary significantly from the levels at the outset. The pre-injection calibre of the arterioles ranged from 42 - 334 µm. The perivascular microinjection investigations were carried out in 8 cats and each concentration of BQ-3020 *per se*, BQ-123 *per se* and BQ-123 and ET-1 was examined in at least 4 cats. The investigations utilising the intracarotid infusion of BQ-3020 were performed in 3 additional cats.

Perivascular Microapplication of BQ-123 per se

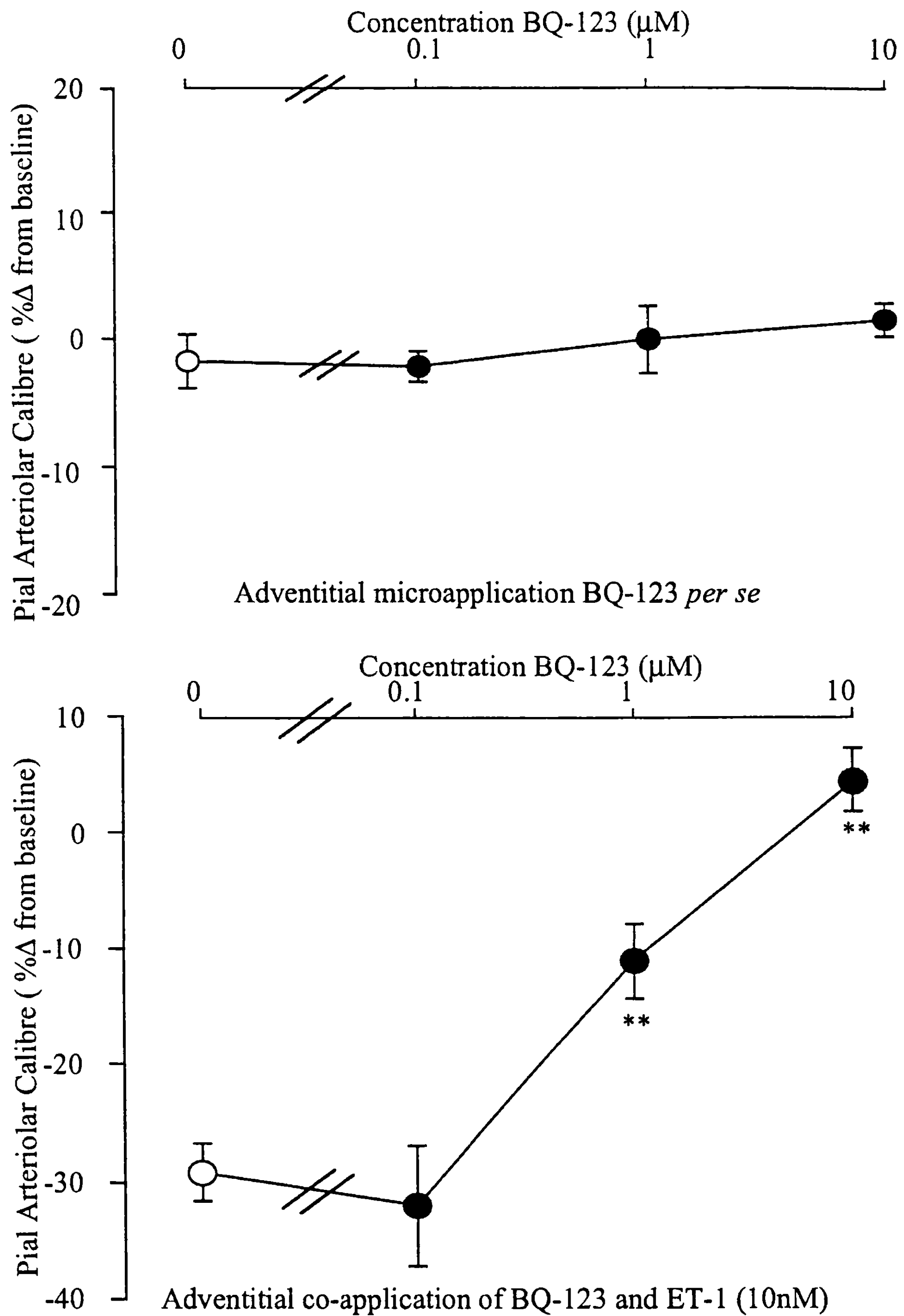
The adventitial microapplications of CSF (pH 7.2) had minimal effect on pial arteriolar calibre [-0.93 ± 1.6% from pre injection baseline; n = 14]. Similarly, the adventitial microapplications of BQ-123 *per se* (0.1 - 10 µM) had minimal effect on pial arteriolar calibre [Figure 9].

Perivascular co-application of ET-1 (10 nM) and BQ-123

The adventitial microapplication of ET-1 (10 nM) effected a marked constriction of the pial arterioles examined [-29.1 ± 2.2 % from pre injection baseline; n = 9]. The adventitial co-application of ET-1 (10 nM) and BQ-123 demonstrated a dose dependent attenuation of the ET-1 mediated vasoconstrictive response [Figure 9]. The concentration of BQ-123 that produced a half maximal attenuation (EC_{50}) was estimated to be approximately 0.7 µM.

Perivascular and intravascular administration of BQ-3020

The <u>adventitial</u> microapplication of CSF had minimal effect on arteriolar calibre, whereas, the <u>adventitial</u> microapplication of BQ-3020 (0.001 - 1 µM) effected dose dependent dilatations of pial arterioles examined (EC_{50} 30 nM) [Figure 10]. The <u>intracarotid</u> infusion of 0.9% saline had minimal effect on pial arteriolar calibre or arterial blood pressure. The magnitude of the response of arterioles to BQ-3020 (100



<u>Figure 9</u>(Top) Vasomotor responses of pial arterioles to the adventitial microapplication of BQ-123 *per se*. There were no significant alterations in the pial arteriolar calibre at any concentration of BQ-123 examined. Data are expressed as percent alteration from baseline of pial arteriolar calibre. Data are presented as mean ± S.E.M (n, number of arterioles examined = 6 for each concentration).

(Bottom) Vasomotor responses of pial arterioles to adventitial co-application of endothelin-1 (10 nM) and BQ-123. BQ-123 significantly attenuated the endothelin-1 induced vasoconstrictions (** $P < 0.01$ for the comparison with endothelin-1 alone). Data are expressed as percent alteration from baseline of pial arteriolar calibre. Data are presented as mean ± S.E.M (n, number of arterioles examined = 5-12 for each concentration).

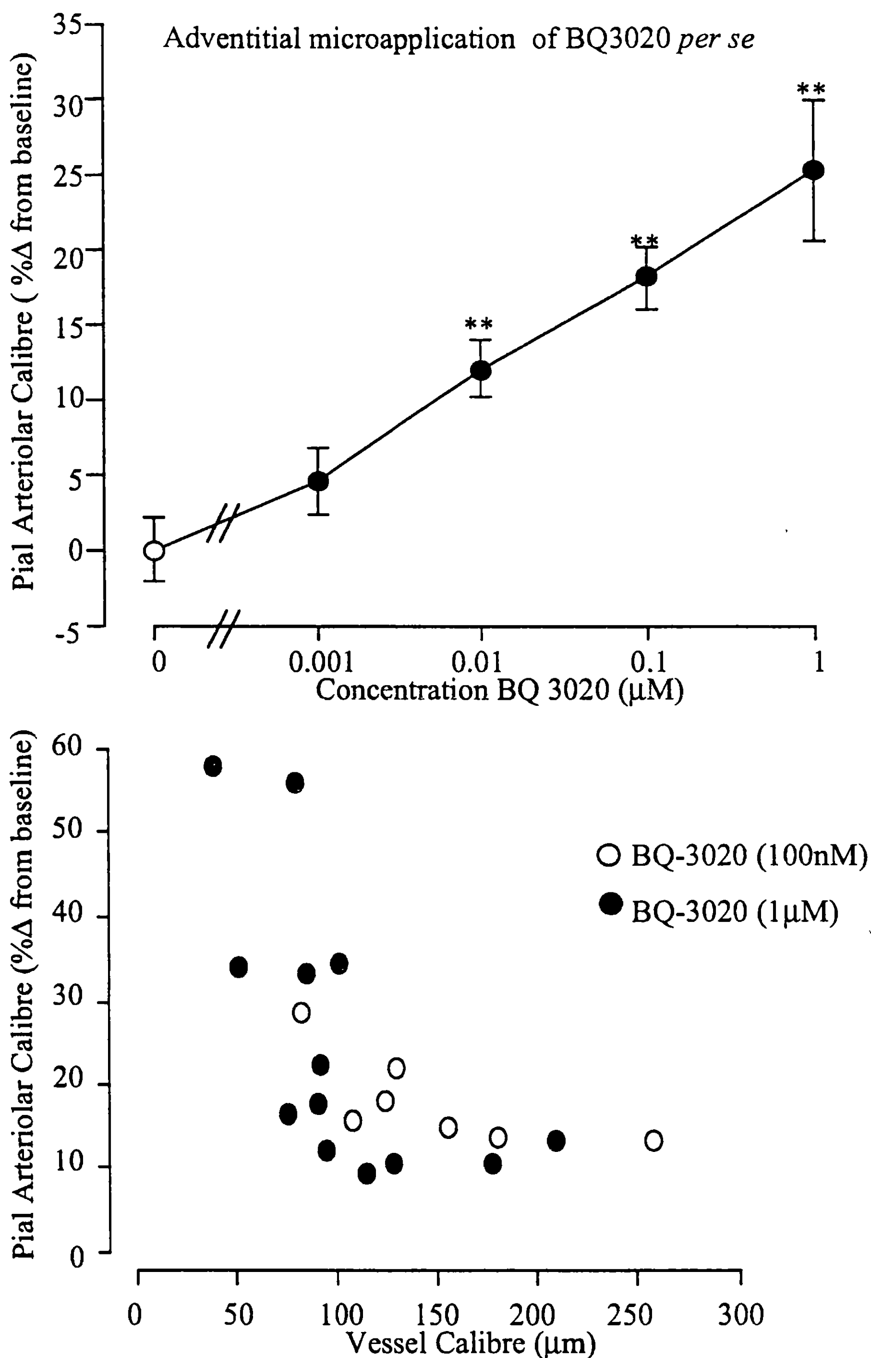


Figure 10(Top) Vasomotor responses of pial arterioles to the adventitial microapplication of BQ-3020 *per se*. BQ-3020 effected a dose dependent vasodilatation of the pial arterioles examined (** $P < 0.01$ for the comparison with CSF). Data are expressed as percent alteration from baseline of pial arteriolar calibre. Data are presented as mean ± S.E.M (n, number of arterioles examined = 7-13 for each concentration).

(Bottom) The dependence of the pial arteriolar dilatation elicited by BQ-3020 on the calibre of arteriole examined. Pial arteriolar diameter was determined prior to the adventitial microapplication of BQ-3020. The arteriolar responses are expressed as the percent change from pre-injection baseline. (r^2= 0.36; $P < 0.01$).

nM and 1μM) was dependent on the pre-injection calibre of the arteriole examined. Cerebral arterioles less than 100 μm demonstrated an increased reactivity to BQ-3020 [Figure 10]. No such correlation was demonstrated with the arterioles to which CSF (pH 7.2) was applied. The intracarotid infusion of BQ-3020 (0.5 - 500 pmol/min) had minimal effect on pial arteriolar calibre [Figure 11]. The intracarotid infusion of BQ-3020 at a rate of 500 pmol/min elicited a transient decrease in mean arterial blood pressure (16.3 ± 8.6 mmHg from pre infusion baseline) and the blood pressure returned to baseline within 5 min.

Feline cerebral arterioles *in situ*

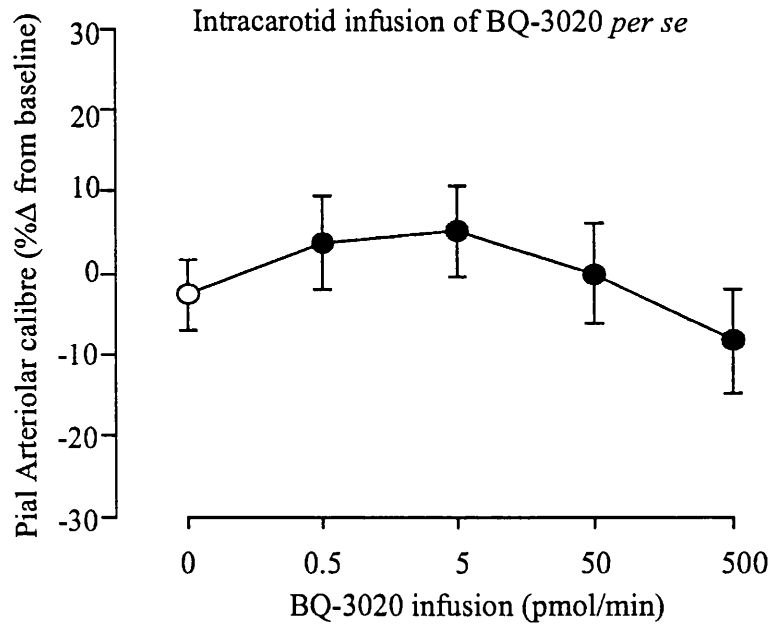


Figure 11 Vasomotor responses of pial arterioles to the intracarotid infusion of BQ-3020. BQ-3020 or saline had minimal effect on pial arterioles examined. Data are expressed as percent alteration from baseline of pial arteriolar calibre. Data are presented as mean ± S.E.M (n, number of arterioles examined = 11 for each concentration).

3.2 Evaluation of Endothelin Receptor Antagonists in the Cerebrovasculature of the Cat

3.2.1 Cerebrovascular effects of Bosentan *in vivo*

At the outset of the study of pial arteriolar responses mean arterial blood pressure was 85 ± 3 mmHg, arterial carbon dioxide tension was 32.0 ± 0.9 mmHg and arterial oxygen tension was 195 ± 6 mmHg. During the course of the study, these physiological variables did not change significantly from the levels at the outset. The pre-injection calibre of the arterioles ranged from 58-319 μm. The perivascular microinjection investigations were carried out in 5 cats and each concentration of Bosentan *per se* and Bosentan and ET-1 was examined in every cat. The effects of the intravenous administration of Bosentan on the perivascular effects of ET-1 were examined in the same 5 cats.

Perivascular Microapplication of Bosentan

The perivascular microapplication of artificial CSF minimally altered pial arteriolar calibre (alteration in calibre 0.8 ± 1.7%, n=7). Perivascular microapplication of Bosentan in the range 0.3 to 300 μM had minimal effect on pial arteriolar calibre when compared to CSF injections [Figure 12].

Perivascular co-Application of Bosentan and Endothelin-1

The perivascular microapplication of endothelin-1 (10 nM) resulted in a marked constriction of pial arterioles (calibre reduced from pre injection baseline by 39.2 ± 2.7%, n=13). Perivascular co-administration of Bosentan with endothelin-1 (10 nM) produced a dose-dependent attenuation of endothelin-induced contractions; the concentration of Bosentan in the co-injectate which would have produced half maximal inhibition was estimated to be approximately 1 μM [Figure 12]. Perivascular microapplication of alkalotic CSF (pH 7.45; HCO_3^- 22 mM) effected significant reductions in pial arteriolar calibre compared to CSF alone (calibre reduced from pre

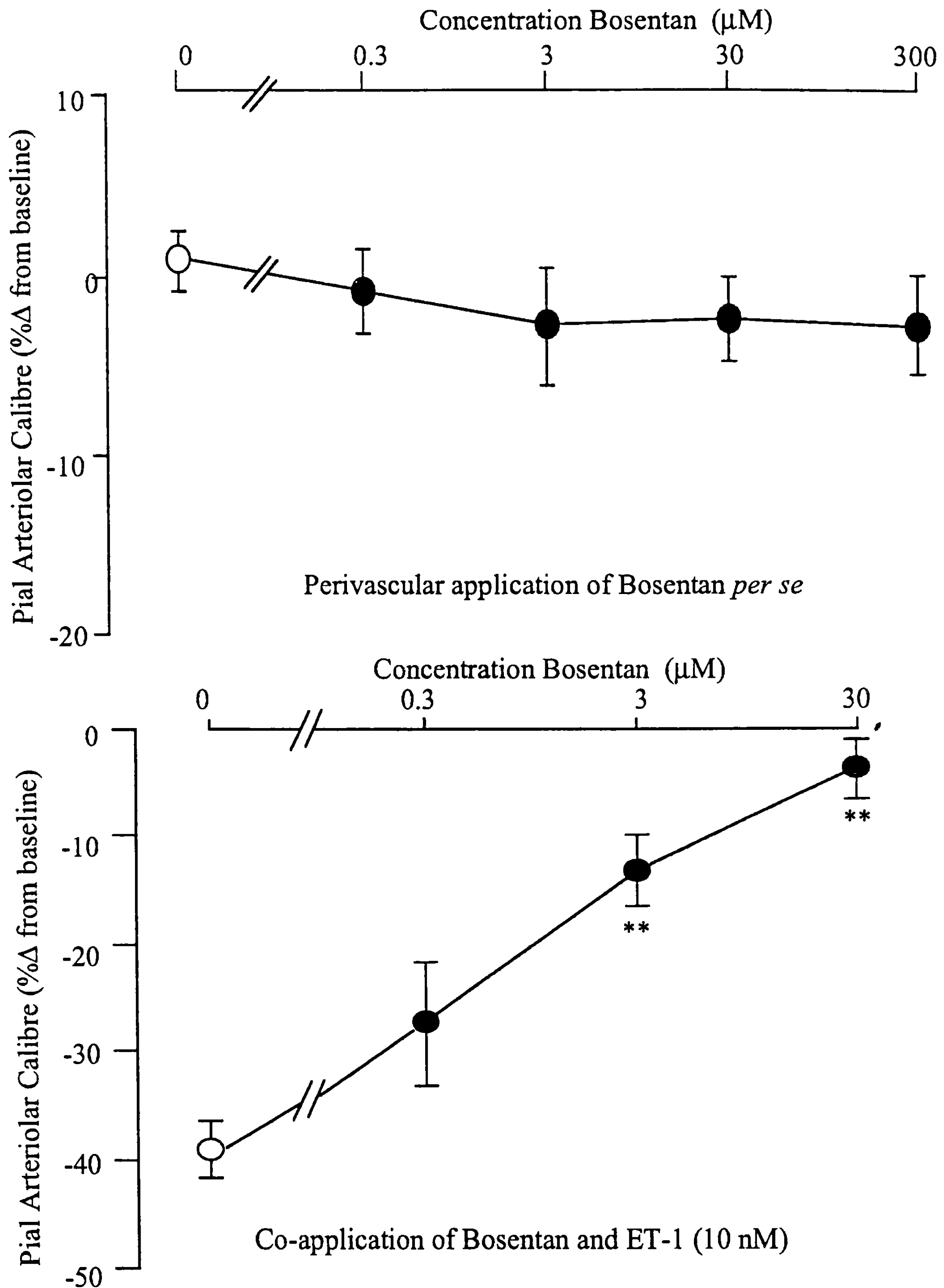


Figure 12 (Top) Vasomotor responses of pial arterioles to perivascular microapplication of bosentan. There were no significant alterations in pial arteriolar calibre at any concentration of bosentan examined. Data are expressed as the percent alteration from baseline in pial arteriolar calibre. Data are presented as mean ± S.E.M (n, number of arterioles examined = 7-11 for each concentration).

(Bottom) Vasomotor responses of pial arterioles to perivascular co-application of endothelin-1 (10nM) and bosentan. Bosentan significantly attenuated the constrictions induced by endothelin-1. (**$P<0.01$ for the comparison with endothelin alone.). Data are expressed as the percent alteration from baseline in pial arteriolar calibre. Data are presented as mean ± S.E.M (n, number of arterioles examined = 7-13 for each concentration).

injection baseline by 16.6 ± 2.7 %, n= 7). Alkalotic CSF containing Bosentan (30 μM) effected a reduction in pial arteriolar calibre of a similar magnitude to alkalotic CSF (pH 7.45; HCO_3^-22 mM) (calibre reduced from pre injection baseline by 14.9 ± 1.9 %, n= 7).

Reversal of ET-1 induced vasoconstrictions

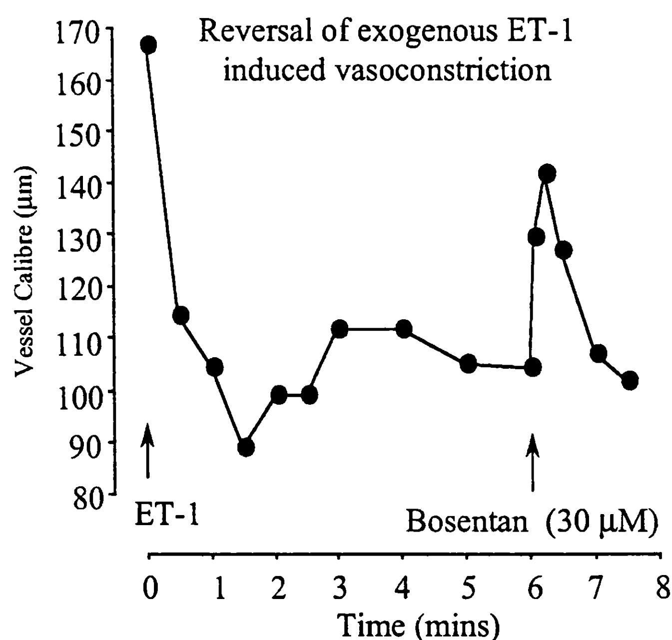
Normal pial arterioles were constricted (-29.1 ± 1.9% from baseline; n = 12) by the perivascular microapplication of endothelin-1 (10 nM). Bosentan (30 μM) or CSF (pH 7.2) was applied to these vessels 5-15 minutes following the application of endothelin-1. Bosentan (30 μM) effected a marked increase in vessel calibre from the ET-1 constricted baseline (22.4 ± 3.6% from baseline ; n = 7; $P < 0.01$ for the comparison with the effects of CSF (pH 7.2)) [Figure 13]. The reversal or dilatation was transient with a typical duration of approximately 30 seconds before the vessel returned to pre Bosentan injection calibre [Figure 13]. The application of CSF on these constricted vessels had minimal effect on the vessel calibre (-1.9 ± 1.8% from ET-1 constricted baseline ; n = 6) [Figure 13].

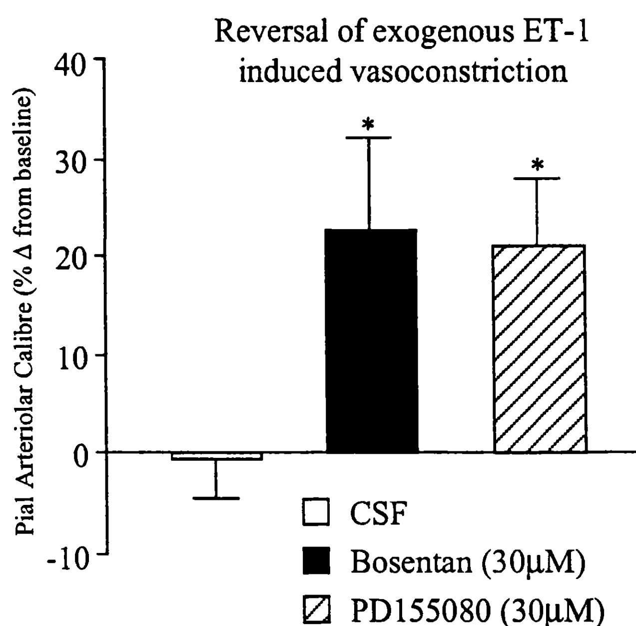
Perivascular Microapplication of Endothelin 1 after Intravenous Administration of Bosentan

The intravenous administration of Bosentan (17 μmol/kg) had minimal effect on mean arterial blood pressure. Perivascular microapplication of endothelin-1 (10 nM) in the period 15-75 min.. after the ***intravenous*** injection of Bosentan (17 μmol/kg) produced minimal alterations in pial arteriolar calibre [Figure 14]. The significant constrictions produced by perivascular microapplication of alkalotic CSF (pH 7.45) were not influenced by the intravenous injection of Bosentan [Figure 14].

Subarachnoid microapplications of autologous arterial blood produce immediate marked significant reductions in pial arteriolar calibre; the acute constrictions produced by microapplications of blood were not influenced by the intravenous injection of Bosentan [Figure 14].

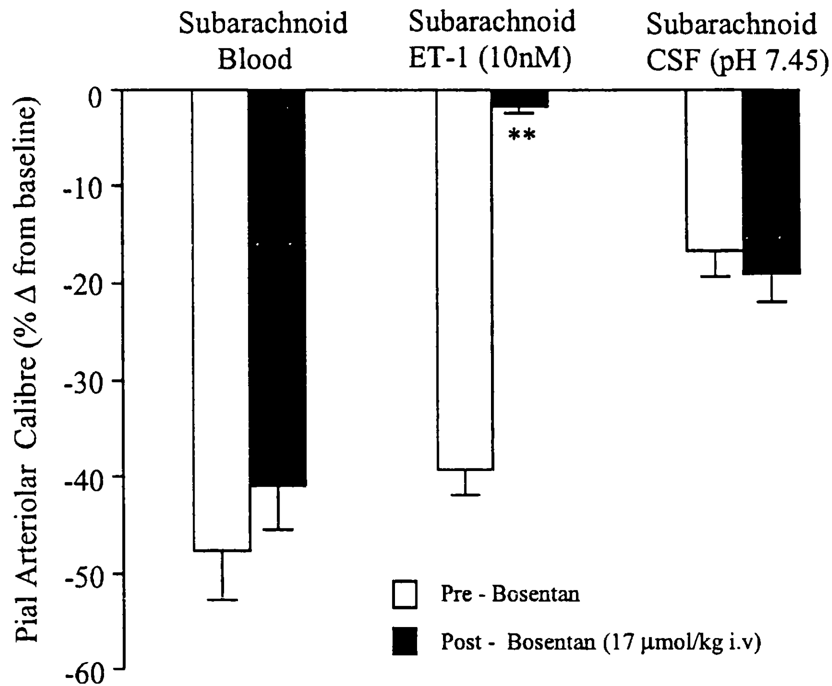
Feline cerebral arterioles *in situ*





<u>Figure 13</u> (Top) Vasomotor response of a normal pial arteriole to the perivascular microapplication of ET-1 (10 nM). Perivascular microapplication of Bosentan (30 µM) elicited a transient reversal of the ET-1 induced vasoconstriction.

(Bottom) Reversal of exogenous ET-1 (10 nM) induced vasoconstriction of normal pial arterioles. Microinjections of CSF, Bosentan (30 µM) and PD155080 (30 µM) were carried out 5 -15 min following the microapplication of ET-1 (10 nM). Data are presented as mean ± S.E.M percent change from pre-injection baseline (n= 7-12 in each group). * $P<0.01$ for the comparison between ET-1 and Bosentan or PD155080



<u>**Figure 14**</u> Vasomotor responses of pial arterioles to subarachnoid perivascular microapplications of endothelin-1 (10 nM), blood or alkalotic CSF (7.45) after the intravenous injection of bosentan (17μmol/kg). The pial arteriolar constrictions induced by endothelin-1 were significantly attenuated by intravenous bosentan whereas the constrictions induced by subarachnoid blood or alkalotic CSF were minimally affected (**$P<0.01$ for the comparison with endothelin alone).Data are expressed as the percent alteration from baseline in pial arteriolar calibre. Data are presented as mean ± S.E.M (n, number of arterioles examined = 7-13 for each concentration).

3.2.2 Cerebrovascular effects of PD155080 *in vivo*

At the outset of the study of pial arteriolar responses mean arterial blood pressure was 93 ± 3 mmHg, arterial pH was 7.4 ± 0.01, arterial carbon dioxide tension was 31.5 ± 0.7 mmHg and arterial oxygen tension was 206 ± 6 mmHg. During the course of the study, these physiological variables did not change significantly from the levels at the outset. The pre-injection calibre of the arterioles ranged from 56-334 μm. The perivascular microinjection investigations were carried out in 4 cats and each concentration of PD155080 *per se* and PD155080 and ET-1 was examined in every cat. The effects of the intravenous administration of PD155080 on the perivascular effects of ET-1 were examined in the same 4 cats. The effects of perivascular choline chloride *per se*, choline chloride and ET-1 and the intravenous choline chloride were examined in 3 separate cats.

Perivascular Microapplication of PD155080

The subarachnoid perivascular microapplication of CSF minimally altered pial arteriolar calibre. Microapplication of PD155080 (0.3-3 μM) did not significantly alter pial arteriolar calibre relative to CSF [Figure 15]. Perivascular microinjection of CSF containing choline chloride (30 μM) did not significantly alter pial arteriolar calibre (mean change in calibre 0.3 ± 2.6%; n=5).

Perivascular co-application of PD155080 and Endothelin-1

The subarachnoid perivascular microapplication of endothelin-1 (10 nM) effected marked constriction of cortical pial arterioles. Co-administration of PD155080 with endothelin-1 resulted in attenuation of the vasoconstrictor effects of endothelin. The IC_{50} value was approximately 1 μM [Figure 15].

Reversal of ET-1 induced vasoconstrictions

The perivascular administration of CSF to pial arterioles constricted with endothelin-1 (5 min. previously) had minimal effect on calibre (mean change in

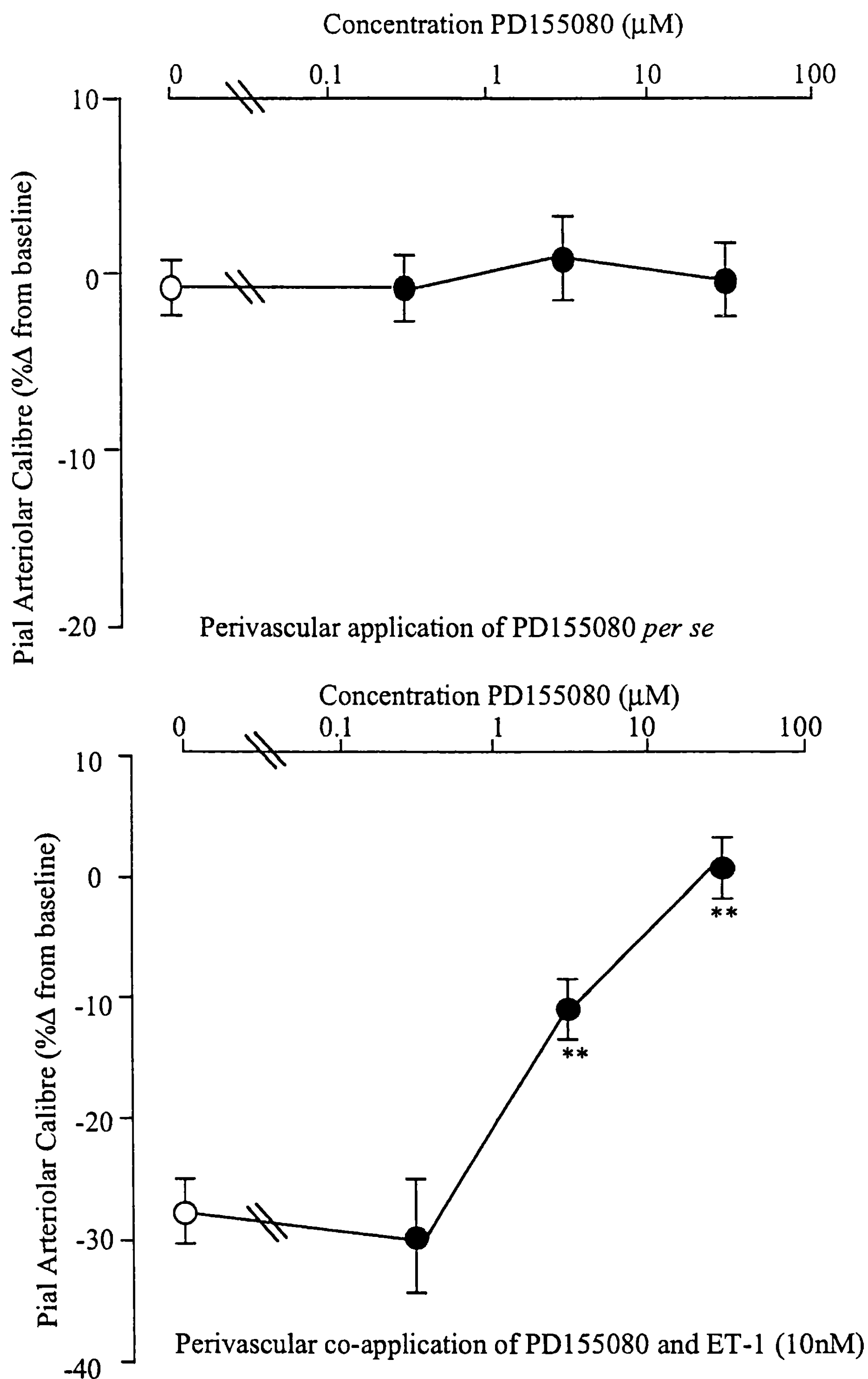


Figure 15 Vasomotor responses of pial arterioles to perivascular microapplication of PD155080, a non-peptide antagonist, (Top) and co-application of endothelin-1 (10 nM) and PD155080 (Bottom). There were no significant alterations in pial arteriolar calibre at any concentration of PD155080 examined. PD155080 significantly attenuated the constrictions induced by endothelin-1. (**$P<0.01$ for the comparison with endothelin alone.). Data are expressed as the percent alteration from baseline in pial arteriolar calibre. Data are presented as mean ± S.E.M (n, number of arterioles examined = 6-13 for each concentration).

calibre from pre-CSF injection 0.8 ± 1.4%; n=6) [Figure 13]. The perivascular administration of PD155080 (30μM) to a pial arteriole constricted with endothelin-1 resulted in a significant increase in calibre from the level immediately prior to PD155080 injection (mean change in calibre 21 ± 2.4%; n=8) [Figure 13]. Approximately 5 min. after microapplication of PD155080, the calibre had returned to its pre-drug constricted diameter.

Intravenous administration of PD155080

The intravenous administration of PD155080 (19.2 μmol/kg) produced marked alterations in arterial blood pressure during the course of the injection. The intravenous injection of choline chloride (71.6 μmol/kg) in isotonic saline (vehicle) produced similar alterations in arterial blood pressure. After completion of the injection of either PD155080 or choline chloride, arterial blood pressure returned rapidly to its pre-injection level.

The perivascular microapplication of endothelin-1 (10 nM) in choline chloride treated cats resulted in significant reductions in pial arteriolar calibre [Figure 16]. In contrast, the perivascular microapplication of endothelin-1 had minimal effect on pial arteriolar calibre in the period 15 to 75 min.. after the intravenous injection of PD155080 (19.2 μmol/kg) [Figure 16]. The perivascular microapplication of alkalotic CSF (pH 7.45) resulted in reductions of pial arteriolar calibre of a similar magnitude in cats which received either vehicle or intravenous PD155080 [Figure 17].

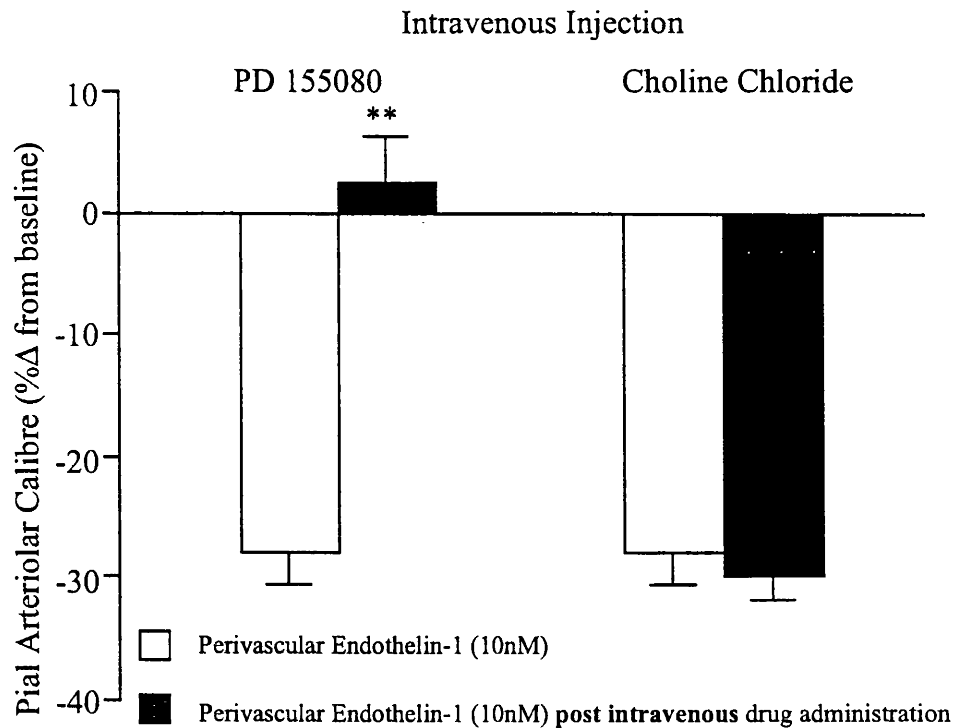


Figure 16 Vasomotor responses of pial arterioles to perivascular microapplications of endothelin-1 (10 nM) after the intravenous injection of PD155080 (19.2 μmol/kg) or choline chloride (72 μmol/kg). The pial arteriolar constrictions induced by endothelin-1 were significantly attenuated by intravenous PD155080 and minimally affected by intravenous choline chloride (**$P<0.01$ for the comparison with endothelin alone). Data are expressed as the percent alteration from baseline in pial arteriolar calibre. Data are presented as mean ± S.E.M (n, number of arterioles examined = 6-12 in each group).

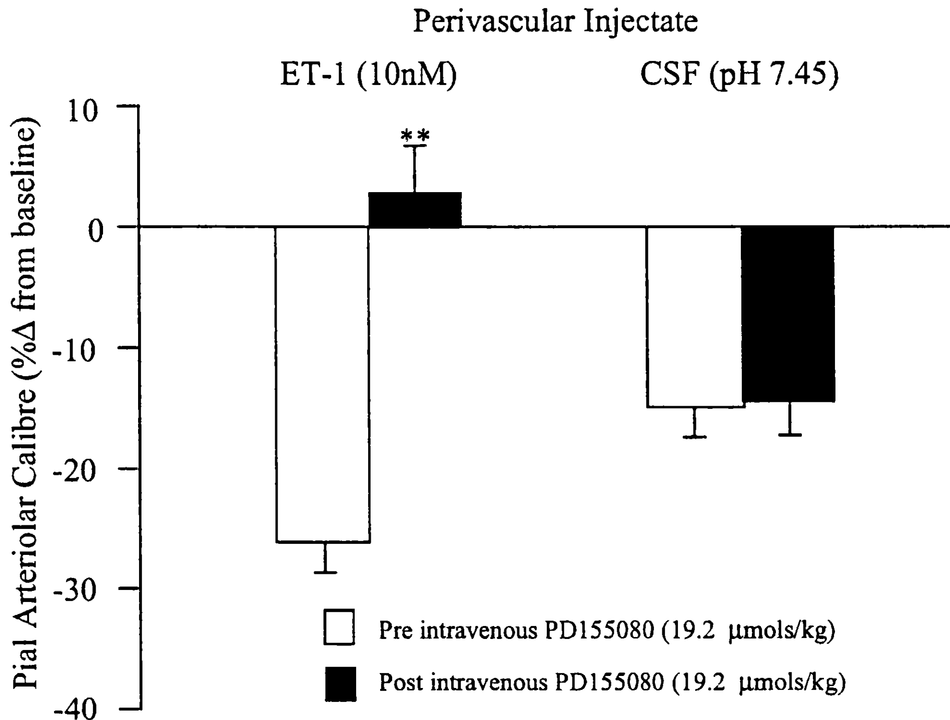


Figure 17 Vasomotor responses of pial arterioles to perivascular microapplications of endothelin-1 (10 nM) or alkalotic CSF (pH 7.45) after the intravenous injection of PD155080 (19.2 μmol/kg). The pial arteriolar constrictions induced by endothelin-1 were significantly attenuated by intravenous PD155080 whereas the constrictions induced by alkalotic CSF were minimally affected (**$P<0.01$ for the comparison with endothelin alone). Data are expressed as the percent alteration from baseline in pial arteriolar calibre. Data are presented as mean ± S.E.M (n, number of arterioles examined = 6-12 in each group).

3.2.3 Cerebrovascular effects of PD145065 *in vivo*

At the outset of the study of pial arteriolar responses mean arterial blood pressure was 92 ± 2 mmHg, arterial pH was 7.42 ± 0.01, arterial carbon dioxide tension was 30.7 ± 0.7 mmHg and arterial oxygen tension was 225 ± 4 mmHg. During the course of the study, these physiological variables did not change significantly from the levels at the outset. The pre-injection calibre of the arterioles ranged from 38-283 μm. The perivascular microinjection investigations were carried out in 6 cats and each concentration of PD145065 *per se* and PD145065 and ET-1 was examined in at least 4 of the cats. The effects of the intravenous administration of PD145065 on the perivascular effects of ET-1 were examined in at least 4 cats.

Perivascular Microapplication of PD145065

The subarachnoid perivascular microapplication of CSF minimally altered pial arteriolar calibre. Microapplication of PD145065 (0.03-3 μM) had minimal effect on pial arteriolar calibre when compared to CSF injections [Figure 18].

Perivascular co-Application of PD145065 and Endothelin-1

The subarachnoid perivascular microapplication of endothelin-1 (10 nM) effected marked constriction of cortical pial arterioles. Co-administration of PD145065 with endothelin-1 resulted in a dose dependent attenuation of the vasoconstrictor effects of endothelin. The IC_{50} value was approximately 0.1 μM [Figure 18].

Perivascular Microapplication of Endothelin 1 after Intravenous administration of PD145065

The intravenous administration of PD145065 (30 μmol/kg) had minimal effect on mean arterial blood pressure. The perivascular microapplication of ET-1 (10 nM) resulted in a marked reduction in arteriolar calibre. The intravenous administration of PD145065 (30 μmol/kg) demonstrated no significant attenuation of the

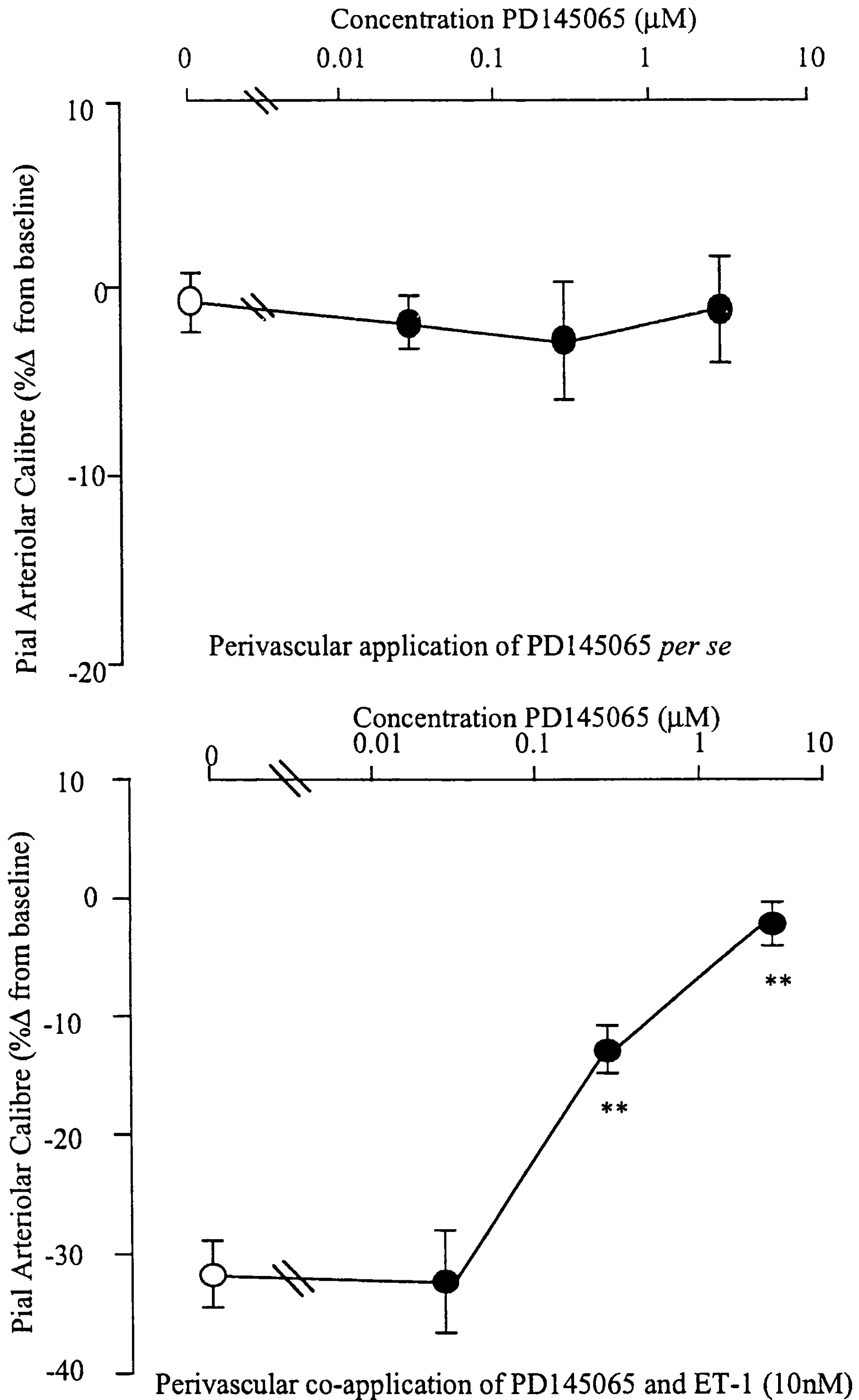


Figure 18 Vasomotor responses of pial arterioles to perivascular application of PD145065 a peptide antagonist (Top) and co-application of endothelin-1 (10 nM) and PD145065 (Bottom). There were no significant alterations in pial arteriolar calibre at any concentration of PD145065 examined. PD145065 significantly attenuated the constrictions induced by endothelin-1. (**P<0.01 for the comparison with endothelin alone.). Data are expressed asthe percent alteration from baseline in pial arteriolar calibre. Data are presented as mean ± S.E.M (n, number of arterioles examined = 6-25 for each concentration).

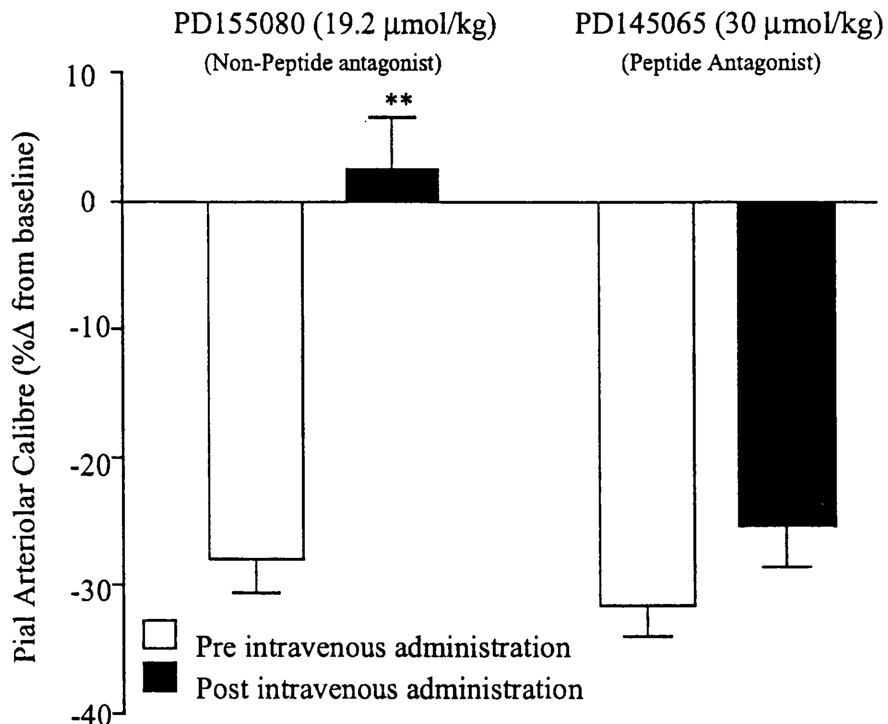


Figure 19 Vasomotor responses of pial arterioles to perivascular microapplications of endothelin-1 (10 nM) after the intravenous injection of PD155080 (19.2 µmol/kg) or PD145065 (30 µmol/kg). The pial arteriolar constrictions induced by endothelin-1 were significantly attenuated by intravenous PD155080 but minimally affected following the intravenous administration of PD145065 (**$P<0.01$ for the comparison with endothelin alone). Data are expressed as the percent alteration from baseline in pial arteriolar calibre. Data are presented as mean ± S.E.M (n, number of arterioles examined = 8-25 in each group).

vasoconstrictive response as a result of the perivascular microapplication of ET-1 indicating the failure of the peptide endothelin receptor antagonist to gain access to the cerebrovascular smooth muscle [Figure 19].

The adequacy of the dose of PD145065 used and its antagonistic activity in the periphery was examined by administering an intravenous bolus of ET-1 (1 nmol/kg) at 120 min. following the initial intravenous administration of PD145065. There was a marked attenuation of both the depressor and pressor responses to ET-1 demonstrating that the dose of PD145065 used significantly inhibited the peripheral effects of systemic ET-1 administration [Figure 20].

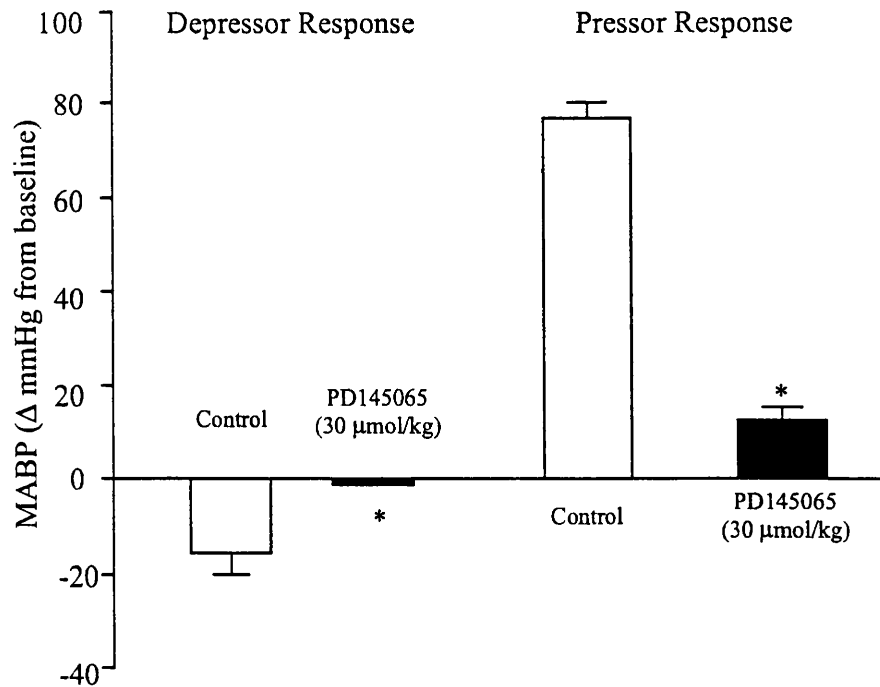


Figure 20 Alterations in the mean arterial blood pressure (MABP) in cat following the intravenous administration of endothelin-1 (1 nmol/kg). In a separate group of animals ET-1 (1 nmol/kg) was administered 120 min. following the intravenous administration of PD145065 (30 µmol/kg). The pressor and depressor responses induced by endothelin-1 were significantly attenuated by intravenous PD145065 (*$P<0.05$ for the comparison with endothelin alone). Data are expressed as the alteration in MABP from baseline . Data are presented as mean ± S.E.M ($n = 4$ animals in each group).

3.3 Pathophysiologic Significance of Endothelins in the Cerebral Circulation

3.3.1 Cerebral Blood Flow following Bilateral Common Carotid Occlusion with Concomitant Haemorrhagic Hypotension in the Rat

Cardiovascular and Respiratory Variables

Hydrogen clearance: There were no significant differences in the cardiovascular and respiratory variables in either the drug or vehicle treated animals following bilateral common carotid artery occlusion and systemic hypotension. The mean arterial blood pressure (MABP) at the outset was 97 ± 1 mmHg in vehicle treated animals and 102 ± 2 mmHg in the Bosentan treated animals [Figure 21]. Arterial pCO_2 was 39.5 ± 1 mmHg (vehicle group) and 39.2 ± 1 (Bosentan group) at the outset of the experiment and did not vary significantly from these levels [Table 5]. The mean rectal temperature was 37.0 ± 0.1°C (vehicle group) and 36.9 ± 0.1°C (Bosentan group) and did not vary significantly from these levels [Table 5]. The MABP was maintained between 42 - 50 mmHg during the period of occlusion [Figure 21]. On the release of the ligatures around the carotid arteries, the blood pressure was restored to pre occlusion levels by a slow intravenous infusion of blood. The administration of Bosentan (17 µmol/kg) had minimal effect on mean arterial blood pressure.

Autoradiography: There were no significant differences in the cardiovascular and respiratory variables in either the drug or vehicle treated animals subjected to bilateral common carotid artery occlusion and systemic hypotension. The mean arterial blood pressure was maintained at 46 ± 1 mmHg (Bosentan) and 46 ± 2 mmHg (vehicle) during the period of occlusion [Table 6]. Prior to start of the blood flow determination, MABP was 95 ± 3 mmHg (Bosentan) and 105 ± 5 mmHg (vehicle) [Table 6]. Arterial pCO_2 was 40.1 ± 0.9 mmHg (Bosentan) and 39.8 ± 0.6 mmHg (vehicle) prior to the start of the blood flow determination [Table 6].

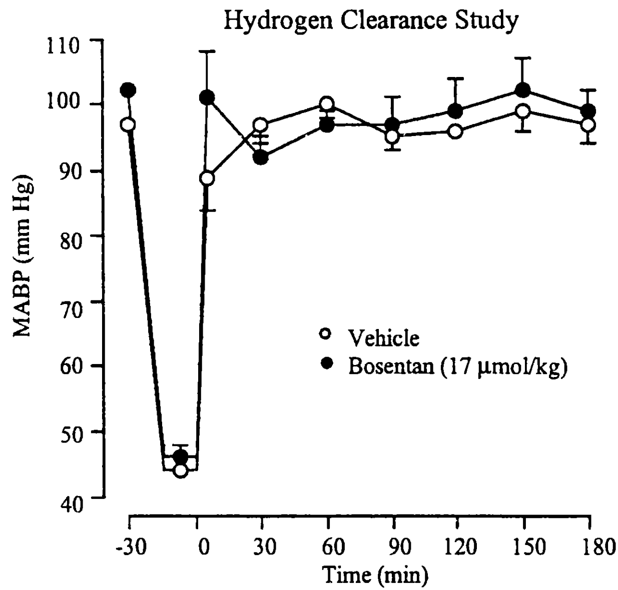


Figure 21 Mean arterial blood pressure in animals subjected to transient bilateral common carotid artery occlusion and systemichypotension (hydrogen clearance study). Bosentan (17 µmol/kg)was administered as an intravenous bolus 10 min. prior to the induction of ischaemia. Results are expressed as mean ± S.E.M (n = 6 in each group).

Table 5

Transient Bilateral Common Carotid Occlusion with Concomitant Haemorrhagic Hypotension

Physiologic Variables: Hydrogen Clearance study

	pH		pCO_2 (mmHg)		pO_2 (mmHg)		Plasma Glucose (mM)		Rectal Temperature (^{O}C)	
	Vehicle	**Bosentan**	**Vehicle**	**Bosentan**	**Vehicle**	**Bosentan**	**Vehicle**	**Bosentan**	**Vehicle**	**Bosentan**
Pre-Occlusion	7.41 ± 0.01	7.43 ± 0.01	40 ± 1	39 ± 1	132 ± 3	138 ± 3	10.9 ± 0.4	9.7 ± 0.4	36.8 ± 0.08	36.9 ± 0.07
Occlusion	7.42 ± 0.01	7.41 ± 0.01	33 ± 2	33 ± 2	156 ± 4	156 ± 11			37.2 ± 0.09	37.1 ± 0.08
5mins	7.33 ± 0.01	7.32 ± 0.01	38 ± 2	37 ± 2	158 ± 10	159 ± 16			36.5 ± 0.10	36.5 ± 0.13
30 mins	7.39 ± 0.01	7.32 ± 0.01	39 ± 1	40 ± 1	144 ± 4	137 ± 6	12.5 ± 0.8	12.2 ± 0.6	36.9 ± 0.27	37.0 ± 0.14
60 mins	7.41 ± 0.01	7.40 ± 0.01	39 ± 1	40 ± 1	143 ± 4	133 ± 5			37.1 ± 0.15	37.0 ± 0.08
90 mins	7.40 ± 0.01	7.41 ± 0.02	39 ± 1	40 ± 1	146 ± 6	143 ± 3			37.0 ± 0.07	36.9 ± 0.09
120 mins	7.39 ± 0.01	7.40 ± 0.01	38 ± 1	39 ± 1	146 ± 3	158 ± 11			37.0 ± 0.04	37.0 ± 0.07
150 mins	7.38 ± 0.01	7.39 ± 0.01	40 ± 1	39 ± 1	142 ± 9	146 ± 8			37.1 ± 0.08	36.8 ± 0.15
180 mins	7.38 ± 0.02	7.38 ± 0.07	39 ± 1	39 ± 1	139 ± 8	143 ± 3			36.9 ± 0.09	37.0 ± 0.3

Data are expressed as mean ± sem (n = 5 - 6 in each group). There were no significant differences between the vehicle and Bosentan treated groups in any of the parameters measured.

Table 6

Physiologic Variables: [^{14}C] - Iodoantipyrine Autoradiography study

	Transient Bilateral Common Carotid Artery Occlusion with Concomitant Haemorrhagic Hypotension		Transient Bilateral Common Carotid Artery Occlusion	
	Vehicle	**Bosentan**	**Vehicle**	**Bosentan**
pH (mmHg)	7.43 ± 0.01	7.42 ± 0.01	7.43 ± 0.01	7.43 ± .01
pCO_2 (mmHg)	39.8 ± 0.6	40.1 ± 1	40.7 ± 0.4	40.4 ± 0.4
pO_2 (mmHg)	190 ± 13	182 ± 7	188 ± 5	179 ± 9
Rectal Temp. (^0C)	37.0 ± 0.1	37.0 ± 0.1	37.2 ± 0.1	37.0 ± 0.1
MABP Pre-Occ.(mmHg)	100 ± 5	98 ± 3	97 ± 3	92 ± 2
MABP Occlusion (mmHg)	45 ± 1	43 ± 1	110 ± 6	107 ± 7
MABP Pre-CBF (mmHg)	105 ± 5	95 ± 3	95 ± 2	94 ± 2
n	8	9	9	9

Data are expressed as mean ± S.E.M (n = number of animals in each group). There were no significant differences between the vehicle and Bosentan treated groups in any of the parameters measured.

Hydrogen Clearance

In the caudate nucleus, cerebral blood flow (CBF) was reduced by 5 ± 1% (of pre occlusion baseline) on the occlusion of the common carotid arteries and induction of hypotension. A similar drop in cerebral blood flow was obtained in the drug treated animals [5 ± 3% of pre occlusion baseline]. The release of the carotid ligatures resulted in a marked hyperaemic response in both drug and vehicle treated group [160 ± 26% of baseline (Bosentan); 142 ± 30% of baseline (vehicle)]. The post ischaemic hypoperfusion lasted from 30 min to 180 min (end of experimental period). The hypoperfusion phase saw cerebral blood flow reduced by approximately 50% (of pre-occlusion baseline). Pre-treatment with Bosentan did not alter the level of hypoperfusion [Figure 22].

In the parietal cortex the occlusion of the common carotid arteries and induction of haemorrhagic hypotension resulted in similar reductions in CBF in the cerebral cortex [15 ± 5% of pre occlusion baseline (Bosentan); 10 ± 3% of pre occlusion baseline (vehicle)]. On the release of the carotid ligatures a hyperaemic response was measured in animals pre-treated with Bosentan [128 ± 38% of baseline]. However, no such response was measured in vehicle treated animals. The reasons for the differences are unclear but could be attributed to the time of the blood flow measurement immediately following the release of the carotid ligatures. The level of hypoperfusion in the cerebral cortex stabilised after 60 min of reperfusion at approximately 45% of pre occlusion baseline [Figure 22]. There was no alteration in the level of hypoperfusion following the pre-treatment with Bosentan.

Autoradiography

The reduction in cerebral blood flow following transient global cerebral ischaemia is illustrated in figure 23. There were no significant alterations in cerebral blood flow, between animals treated with vehicle or Bosentan, in any of the 35 regions examined [Table 7; Figure 24]. Qualitative assessment of the autoradiograms suggested a rostro- caudal variation in the pattern of cerebral blood flow. In the

Transient bilateral common carotid artery occlusion
with concomitant haemorrhagic hypotension in the rat

Hydrogen Clearance Study

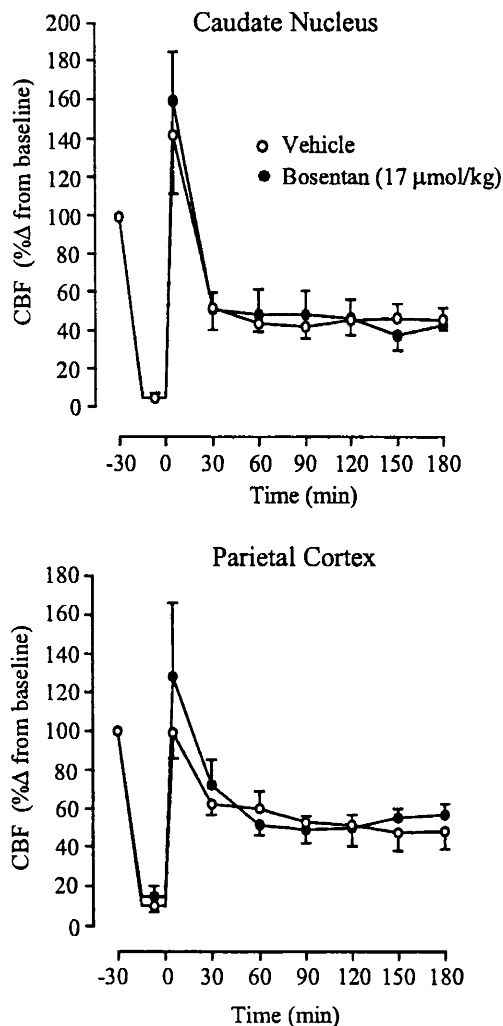


Figure 22(Top)Alterations in cerebral blood flow in the caudate nucleus, determined by hydrogen clearance, in animals subjected to transient bilateral common carotid artery occlusion and systemic haemorrhagic hypotension. Bosentan (17 μmol/kg) was administered as an intravenous bolus 10 min. prior to the induction of ischaemia. Data are expressed as mean ± S.E.M (% of pre-occlusion baseline) (n = 6 in each group).

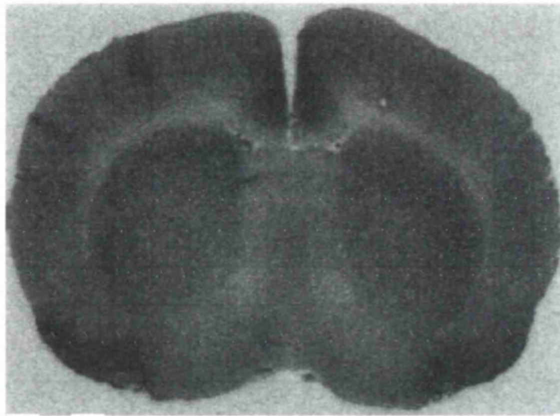
(Bottom) Alterations in cerebral blood flow in the parietal cortex, determined by hydrogen clearance, in animals subjected to transient bilateral common carotid artery occlusion and systemic haemorrhagic hypotension. Bosentan (17 μmol/kg) was administered as an intravenous bolus 10 min. prior to the induction of ischaemia. Data are expressed as mean ± S.E.M (% of pre-occlusion baseline) (n = 6 in each group).

Figure 23 Autoradiograms illustrating the reductions in cerebral blood flow in the caudate nucleus and sensory motor cortex following transient global cerebral ischaemia in the rat. Cerebral blood flow was measured using [^{14}C]-Iodoantipyrine autoradiography at 90 min. following the restoration of blood flow.

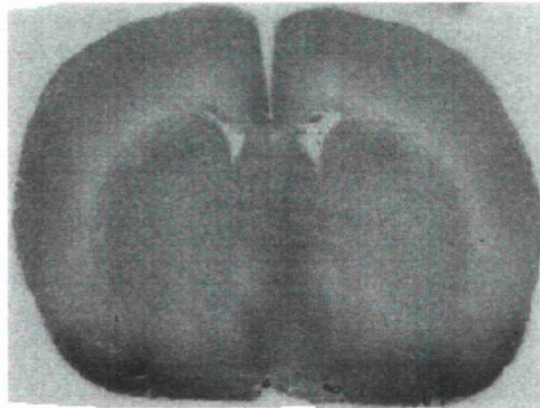
<u>Figure 23</u>

Cerebral Hypoperfusion: Spatial Distribution

Cerebral Blood Flow: ^{14}C-Iodoantipyrine Autoradiography



Sham



Carotid Occlusion with Hypotension

Table 7

Transient Bilateral Common Carotid Artery Occlusion with Concomitant Haemorrhagic Hypotension in the Rat

Region	Vehicle	Bosentan (17 μmol/kg)	t
Cerebellum			
Cerebellar Cortex	109 ± 5	122 ± 7	1.48
Medulla / Pons			
Superior Olivary Nucleus	308 ± 28	362 ± 39	1.10
Pontine gray matter	139 ± 11	160 ± 16	1.05
Mesencephalon			
Inferior Colliculus	238 ± 24	276 ± 23	1.14
Superior Colliculus	168 ± 16	198 ± 20	1.15
Red Nucleus	181 ± 14	207 ± 21	1.00
Substantia Nigra (pars compacta)	119 ± 6	138 ± 10	1.58
Substantia Nigra (pars reticulata)	108 ± 8	121 ± 10	1.57
Diencephalon			
Medial Geniculate body	132 ± 16	165 ± 21	0.99
Sub Thalamic Nucleus	154 ± 14	176 ± 26	0.72
Lateral Geniculate Nucleus	114 ± 23	144 ± 29	0.80
Lateral Habenular Nucleus	180 ± 12	220 ± 22	1.54
Hypothalamus	107 ± 12	107 ± 9	0.00
Mediodorsal Thalamic Nucleus	114 ± 16	132 ± 21	0.67
Ventrolateral Thalamic Nucleus	115 ± 10	151 ± 20	1.55
Anterior Thalamic Nucleus	176 ± 32	222 ± 53	0.72
Telencephalon			
Visual Cortex (layer IV)	132 ± 26	152 ± 34	0.46
Hippocampus (Molecular Layer)	122 ± 19	136 ± 20	0.50
Dentate Gyrus	119 ± 14	143 ± 19	0.99
Auditory Cortex (layer IV)	131 ± 22	153 ± 25	0.65
Posterior Cingulate Cortex	180 ± 38	206 ± 48	0.42
Entorhinal Cortex	133 ± 19	144 ± 23	0.36
Amygdaloid nucleus	92 ± 14	100 ± 16	0.37
Globus pallidus	60 ± 9	68 ± 12	0.52
Caudate Nucleus	77 ± 17	84 ± 21	0.25
Sensory Motor Cortex (layer IV)	95 ± 15	115 ± 19	0.81
Nucleus Accumbens	119 ± 27	127 ± 24	0.22
Anterior Cingulate Cortex	126 ± 36	136 ± 41	0.18
Frontal Cortex	91 ± 14	101 ± 16	0.46
Primary Olfactory Cortex	308 ± 50	290 ± 36	0.30
Parietal Cortex	88 ± 15	98 ± 14	0.49
Myelinated Fibre Tracts			
Cerebellar White Matter	56 ± 4	60 ± 3	0.81
Corpus Callosum	41 ± 5	51 ± 8	1.03
Internal Capsule	44 ± 5	49 ± 4	0.79
Genu	37 ± 8	38 ± 8	0.09

Local cerebral blood flow (ml/100g/min) was measured at 90 min following global ischaemia. Data are expressed as mean ± S.E.M. (n= 8-9 in each group). Two tailed t-test; critical t = 2.13 (15 df) for $P<0.05$. None of the regions examined were significantly different from vehicle.

forebrain regions, e.g. caudate nucleus and sensory motor cortex, a marked reduction in flow was observed in a majority of animals (7/8 vehicle treated; 8/9 Bosentan treated) [Figure 23]. This was in stark contrast to the observations made in more caudal areas of the brain. Reductions in cerebral blood flow were observed in cerebral cortical regions e.g. parietal, visual (7/ 8 (vehicle); 5/ 9 (Bosentan)). The hippocampus showed a greater heterogeneity with CBF appearing to be normal in 3/8 vehicle treated animals and 5/9 Bosentan treated animals. The deep structures of the diencephalon e.g. the medial geniculate body appeared to have "normal" cerebral blood flow in a majority of animals (6/8 vehicle treated; 7/9 Bosentan treated). The differences in the patterns of blood flow may correspond to the variations in the territorial distribution of the arteries supplying the brain.

Transient bilateral common carotid artery occlusion with concomitant haemorrhagic hypotension in the rat: Autoradiography

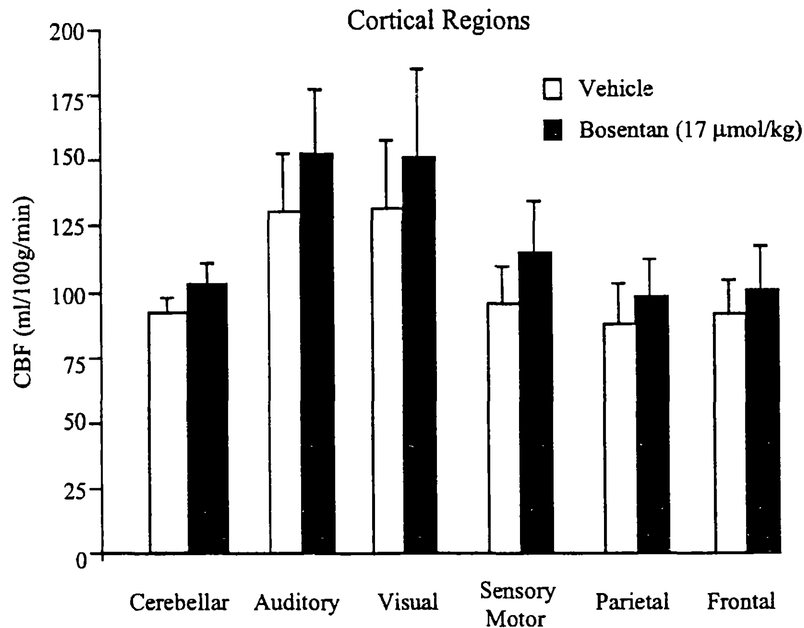


Figure 24 Effect of Bosentan (17 μmol/kg) on cerebral blood flow in the cerebral cortex of animals subjected to transient bilateral common carotid artery occlusion and systemic haemorrhagic hypotension. CBF was measured using ^{14}C-Iodoantipyrine autoradiography at 90 min. following the end of ischaemia. Bosentan was administered as an intravenous bolus 10 min. prior to theinduction of ischaemia. Data are expressed as mean ± S.E.M (ml/100g/min) (n = 8 (vehicle); n=9 (bosentan)).

3.3.1 Cerebral Blood Flow following Bilateral Common Carotid Occlusion in the Rat

Cardiovascular and Respiratory Variables

Hydrogen clearance: There were no significant differences in the cardiovascular and respiratory variables in either the Bosentan or vehicle treated animals subjected to bilateral common carotid artery occlusion. The MABP at the outset was 101 ± 2 mmHg in vehicle treated animals and 103 ± 1 mmHg in the Bosentan treated animals [Table 8; Figure 25]. Arterial pCO_2 was 39.3 ± 0.9 mmHg (vehicle group) and 39.4 ± 0.9 mmHg (Bosentan group) at the outset of the experiment and did not vary significantly from these levels [Table 8]. The mean rectal temperature was 37.1 ± 0.1°C (vehicle group) and 36.8 ± 0.1°C (Bosentan group) and did not vary significantly from these levels [Table 8]. There was a slight increase in the mean arterial blood pressure in animals following to bilateral common carotid artery occlusion. On the release of the ligatures the blood pressure returned to pre occlusion baseline levels. Intravenous administration of Bosentan (17 µmol/kg) had minimal effect on MABP.

Autoradiography: There were no significant differences in the cardiovascular and respiratory variables in either the Bosentan or vehicle treated animals subjected to bilateral common carotid artery occlusion. The mean arterial blood pressure was 106 ± 6 mmHg (Bosentan group) and 108 ± 7 mmHg (vehicle group) during the period of occlusion [Table 6]. Prior to start of the blood flow determination, MABP was 92 ± 2 mmHg (Bosentan group) and 97 ± 3 mmHg (vehicle group) [Table 6]. Arterial pCO_2 was 40.7 ± 0.4 mmHg (Bosentan group) and 40.4 ± 0.4 mmHg (vehicle group) prior to the start of the blood flow determination [Table 6].

Hydrogen Clearance

In the caudate nucleus, the occlusion of the common carotid arteries substantially reduced cerebral blood flow to 58 ± 9% of pre occlusion baseline in

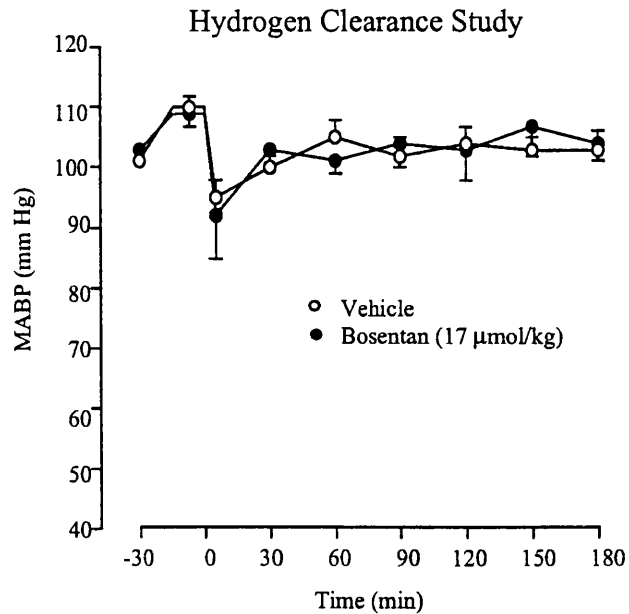


Figure 25 Mean arterial blood pressure in animals subjected to transient bilateral common carotid artery occlusion (hydrogen clearance study). Bosentan (17 µmol/kg) was administered as an intravenous bolus 10 min. prior to the ligation of the common carotid arteries. Data are expressed as mean ± S.E.M (n = 6 in each group).

Table 8

Transient Bilateral Common Carotid Artery Occlusion

Physiologic Variables: Hydrogen Clearance Study

	pH		**pCO_2 (mmHg)**		**pO_2 (mmHg)**		**Plasma Glucose (mM)**		**Rectal Temperature (^{O}C)**	
	Vehicle	**Bosentan**	**Vehicle**	**Bosentan**	**Vehicle**	**Bosentan**	**Vehicle**	**Bosentan**	**Vehicle**	**Bosentan**
Pre-Occlusion	7.41 ± 0.00	7.40 ± 0.01	39 ± 1	39 ± 1	144 ± 8	130 ± 3	10.1 ± 0.5	9.7 ± 0.3	37.1 ± 0.09	36.8 ± 0.08
Occlusion	7.41 ± 0.01	7.40 ± 0.01	39 ± 1	39 ± 1	141 ± 4	140 ± 7			37.1 ± 0.08	36.8 ± 0.08
5mins	7.41 ± 0.01	7.40 ± 0.01	39 ± 1	36 ± 2	143 ± 4	137 ± 8			37.1 ± 0.08	36.9 ± 0.11
30 mins	7.42 ± 0.01	7.40 ± 0.01	37 ± 1	40 ± 1	148 ± 13	133 ± 4	10.2 ± 0.5	10.8 ± 0.4	37.2 ± 0.07	37.0 ± 0.08
60 mins	7.42 ± 0.01	7.39 ± 0.02	39 ± 1	39 ± 1	145 ± 3	135 ± 5			36.9 ± 0.12	36.9 ± 0.07
90 mins	7.41 ± 0.01	7.39 ± 0.02	39 ± 1	39 ± 0.4	138 ± 4	131 ± 7			36.9 ± 0.04	37.1 ± 0.12
120 mins	7.40 ± 0.01	7.40 ± 0.01	39 ± 1	40 ± 1	134 ± 4	129 ± 5			37.0 ± 0.11	37.1 ± 0.09
150 mins	7.39 ± 0.01	7.40 ± 0.01	39 ± 1	39 ± 1	138 ± 3	135 ± 5			37.1 ± 0.22	36.9 ± 0.07
180 mins	7.40 ± 0.01	7.38 ± 0.01	39 ± 1	39 ± 1	140 ± 6	132 ± 7			37.0 ± 0.15	36.9 ± 0.11

Data are expressed as mean ± S.E.M (n = 6 in each group). There were no significant differences between the vehicle and Bosentan treated groups in any of the parameters measured.

Bosentan treated animals and 50 ± 7% of pre occlusion baseline in vehicle treated animals. The release of the carotid ligatures was followed by a small transient hyperaemic response in only the drug treated animals. Cerebral blood flow, in Bosentan treated animals, was elevated by between 111 - 125% of pre occlusion baseline during the first 2 hours of reperfusion [Figure 26]. This increase in blood flow was not statistically significant at any of the time points examined (at 90 min. P = 0.06 (two tailed Student's *t*-test $t = 2.09$; 10 degrees of freedom)).

In the parietal cortex, the occlusion of the common carotid arteries resulted in a reduction of cerebral blood flow [40 ± 6 % of pre occlusion baseline (Bosentan); 41 ± 4 % of pre occlusion baseline (vehicle)]. Cerebral blood flow was elevated in Bosentan treated animals (for the 2 hours post occlusion) whereas it was reduced by 5 - 13% of pre occlusion baseline in the vehicle treated animals [Figure 26]. The small increases in cerebral blood flow were not statistically significant (at 90 min. P= 0.32 (two tailed Student's *t*-test $t = 2.09$; 10 degrees of freedom)).

Autoradiography

There were no significant alterations in cerebral blood flow in either the vehicle or the Bosentan treated animals . There were no significant increases in cerebral blood flow in any of the 35 regions examined [Table 9, Figure 27].

Transient bilateral common carotid artery
occlusion in the rat

Hydrogen Clearance Study

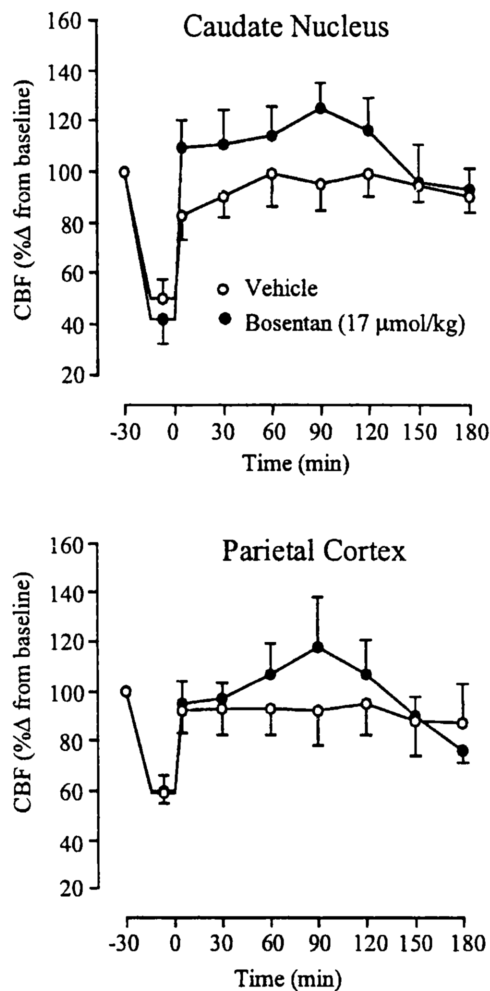


Figure 26 (Top)Alterations in cerebral blood flow in the caudate nucleus, determined by hydrogen clearance, in animals subjected to transient bilateral common carotid artery occlusion. Bosentan (17 μmol/kg) was administered as an intravenous bolus 10 min. prior to the occlusion of the carotid arteries. Data are expressed as mean ± S.E.M (% of pre-occlusion baseline) (n = 6 in each group).

(Bottom)Alterations in cerebral blood flow in the parietal cortex, determined by hydrogen clearance, in animals subjected to transient bilateral common carotid occlusion. Bosentan (17 μmol/kg) was administered as an intravenous bolus 10 min. prior to the ligation of the common carotid arteries. Data are expressed as mean ± S.E.M (% of pre-occlusion baseline) (n = 6 in each group).

Table 9

Transient Bilateral Common Carotid Artery Occlusion in the Rat

Region	Vehicle	Bosentan (17 μmol/kg)	t
Cerebellum			
Cerebellar Cortex	110 ± 4	117 ± 3	1.40
Medulla / Pons			
Superior Olivary Nucleus	300 ± 25	327 ± 23	0.79
Pontine gray matter	155 ± 6	173 ± 10	1.54
Mesencephalon			
Inferior Colliculus	253 ± 17	273 ± 15	0.88
Superior Colliculus	200 ± 8	217 ± 18	0.86
Red Nucleus	207 ± 8	210 ± 13	0.20
Substantia Nigra (pars compacta)	129 ± 4	131 ± 7	0.25
Substantia Nigra (pars reticulata)	112 ± 3	120 ± 9	0.84
Diencephalon			
Medial Geniculate body	192 ± 18	201 ± 19	0.34
Sub Thalamic Nucleus	206 ± 9	214 ± 16	0.44
Lateral Geniculate Nucleus	146 ± 12	175 ± 24	1.08
Lateral Habenular Nucleus	237 ± 15	232 ± 11	0.27
Hypothalamus	116 ± 7	123 ± 8	0.66
Mediodorsal Thalamic Nucleus	162 ± 11	178 ± 15	0.86
Ventrolateral Thalamic Nucleus	161 ± 7	155 ± 7	0.61
Anterior Thalamic Nucleus	200 ± 20	265 ± 30	1.80
Telencephalon			
Visual Cortex (layer IV)	177 ± 13	201 ± 17	1.12
Hippocampus (Molecular Layer)	161 ± 10	181 ± 13	1.22
Dentate Gyrus	157 ± 7	165 ± 8	0.75
Auditory Cortex (layer IV)	219 ± 12	247 ± 26	0.98
Posterior Cingulate Cortex	239 ± 28	284 ± 36	0.99
Entorhinal Cortex	177 ± 11	199 ± 13	1.29
Amygdaloid nucleus	121 ± 7	137 ± 8	1.51
Globus pallidus	102 ± 6	103 ± 6	0.18
Caudate Nucleus	190 ± 6	199 ± 7	0.98
Sensory Motor Cortex (layer IV)	176 ± 8	190 ± 9	1.16
Nucleus Accumbens	167 ± 11	202 ± 14	1.97
Anterior Cingulate Cortex	222 ± 24	317 ± 53	1.63
Frontal Cortex	185 ± 7	193 ± 11	0.61
Primary Olfactory Cortex	331 ± 18	368 ± 32	1.01
Parietal Cortex	170 ± 8	197 ± 16	1.51
Myelinated Fibre Tracts			
Cerebellar White Matter	60 ± 3	64 ± 2	1.11
Corpus Callosum	65 ± 2	65 ± 4	0.00
Internal Capsule	64 ± 3	60 ± 3	0.94
Genu	69 ± 2	77 ± 5	1.49

Local cerebral blood flow (ml/100g/min) was measured at 90 min following carotid occlusion. Data are expressed as mean ± S.E.M. (n= 9 in each group). Two tailed t-test; critical t = 2.12 (16 df) for P<0.05. None of the regions examined were significantly different from vehicle.

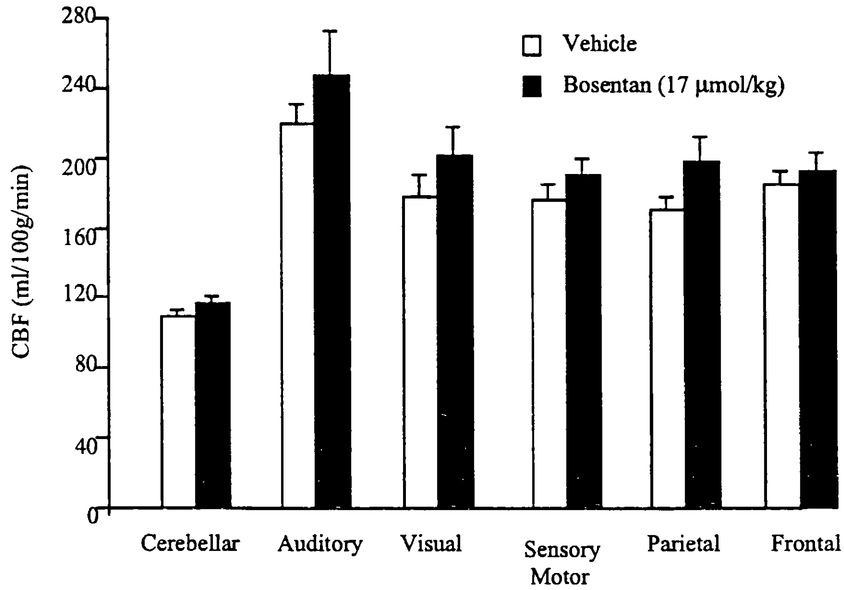


Figure 27 Effect of Bosentan (17 µmol/kg) on cerebral blood flow in the cerebral cortex of animals subjected to transient bilateral common carotidartery occlusion. CBF was measured using ^{14}C-Iodoantipyrine autoradiography at 90 min. following the release of the carotid ligatures.Bosentan was administered as an intravenous bolus 10 min. prior to the ligation of the common carotid arteries. Data are expressed as mean ± S.E.M (ml/100g/min) (n = 9 in each group).

3.3.3 Cerebral Blood Flow following Subdural Haematoma in the Rat

Cardiovascular and Respiratory Variables

The induction of the subdural haematoma in the rat had minimal effect on the cardiovascular and respiratory variables in either the vehicle or Bosentan treated animals [Table 10].

Effect of Bosentan on Cerebral Blood Flow following Subdural Haematoma

The patterns of cerebral blood flow following the induction of the subdural haematoma were assessed by cumulative frequency distribution analysis of cerebral blood flow in the ipsilateral and contralateral hemispheres. This analysis was carried out at eight pre-selected stereotactic levels as described by Gotoh et al., 1986:
Level 1 anterior 10.50 mm; Level 2 anterior 8.92 mm; Level 3 anterior 7.19 mm; Level 4 anterior 6.06 mm; Level 5 anterior 5.15 mm; Level 6 anterior 3.75 mm; Level 7 anterior 2.18 mm; Level 8 anterior 1.02 mm (co-ordinates are relative to intraural line).

The reductions in cerebral blood flow induced by the subdural haematoma were confined to the cerebral cortex [Figure 28]. The severest reductions in cerebral blood flow appeared to be in the cortical regions immediately underlying the haematoma. These observations are reflected in the cumulative frequency distribution curves of cerebral blood flow and area. There were no significant differences in the relationship between the area of tissue and cerebral blood flow in levels 1 to 8 in the vehicle or Bosentan treated animals [Figures 29-36]. There were no significant differences in any of the 25 structures examined [Table 11].

The volumes of tissue in the ipsilateral hemisphere perfused by blood flow of less than 20 ml/100g/min, 20-50 ml/100g/min, 50-100 ml/100g/min and greater than 100 ml/100g/min were calculated from the frequency distribution of blood flow. There was a n apparent decrease in the volume of tissue perfused by less than 20 ml/100g/min in the Bosentan treated animals, although, this reduction was not statistically significant ($t = 0.91$; $P = 0.8$) [Figure 37]. The volumes of tissue perfused

Table 10

Sudural Haematoma in the Rat

Physiologic Variables

	pH		pCO_2 (mmHg)		pO_2 (mmHg)		Plasma Glucose (mM)		Rectal Temperature (^{O}C)	
	Vehicle	**Bosentan**	**Vehicle**	**Bosentan**	**Vehicle**	**Bosentan**	**Vehicle**	**Bosentan**	**Vehicle**	**Bosentan**
Pre SDH	7.44 ± 0.01	7.44 ± 0.01	37.7 ± 0.8	38.2 ± 0.8	192 ± 9	187 ± 11	8.4 ± 0.2	8.5 ± 0.5	37.0 ± 0.1	37.1 ± 0.1
30 min	7.40 ± 0.01	7.42 ± 0.01	39.7 ± 0.7	39.0 ± 1.3	187 ± 4	190 ± 11	9.5 ± 0.5	9.4 ± 1.1	37.2 ± 0.1	37.0 ± 0.1
60 min	7.39 ± 0.02	7.41 ± 0.01	38.8 ± 0.9	38.3 ± 1.3	190 ± 5	195 ± 13			36.9 ± 0.2	37.1 ± 0.2
120 min	7.37 ± 0.02	7.39 ± 0.01	39.2 ± 1.8	37.7 ± 1.0	187 ± 8	169 ± 20			36.9 ± 0.2	37.1 ± 0.1
150 min	7.38 ± 0.01	7.36 ± 0.02	40.0 ± 0.7	39.3 ± 0.8	191 ± 5	178 ± 15			37.0 ± 0.1	37.2 ± 0.3

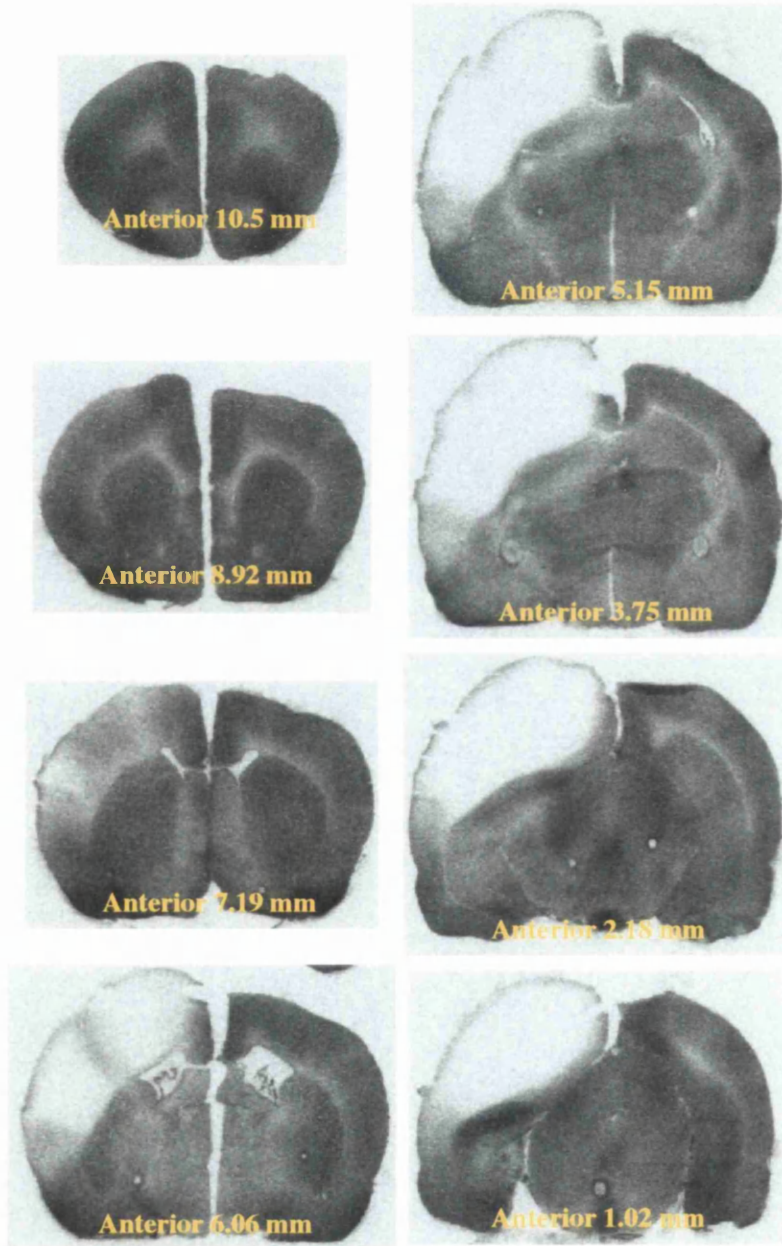
Data are expressed as mean ± sem (n = 6 in each group). The arterial blood samples were taken prior to the induction of the subdural haematoma (SDH) and at the intrvals post-SDH indicated. There were no significant differences between the vehicle and Bosentan treated groups in any of the parameters measured.

Figure 28 Representative autoradiograms, from a single animal, illustrating the marked reductions in cerebral blood flow in the cortex (white areas) and swelling of the ipsilateral hemisphere at 2.5 hours following subdural haematoma in the rat. Cerebral blood flow was measured using [^{14}C]-Iodoantipyrine autoradiography. The stereotaxic co-oridnates are taken relative to the intra-aural line.

Figure 28

Subdural Haematoma in the Rat

Alterations in Cerebral Blood Flow



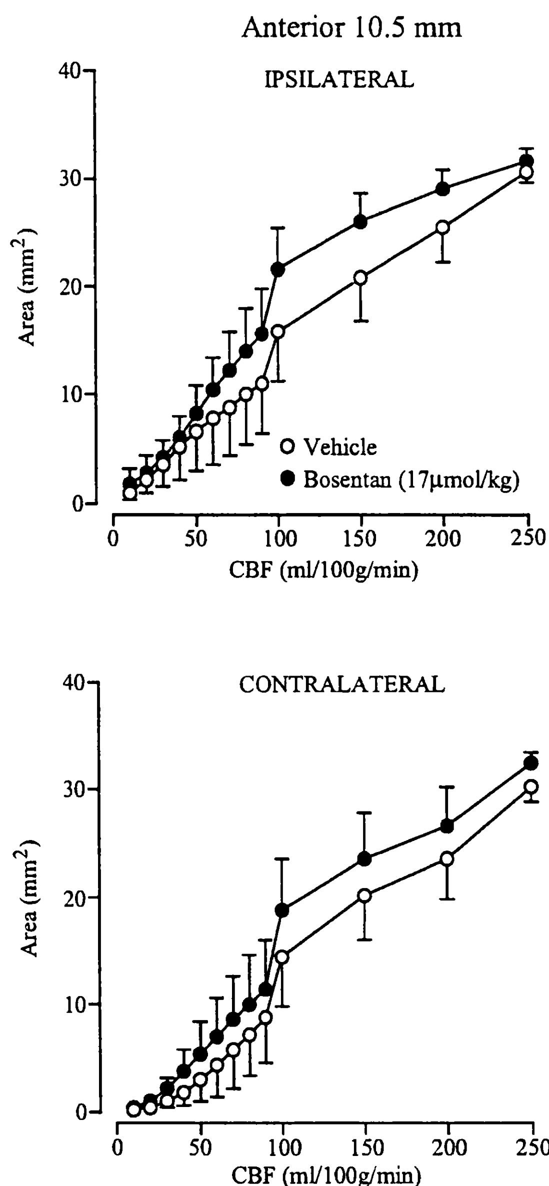


Figure 29 Effects of Bosentan (17 μmol/kg) on cerebral blood flow, determined by frequency distribution analysis, following subdural haematoma. Bosentan (17 μmol/kg) was administered 10 min. prior to the induction of the haematoma and CBF measured 150 min. post-induction of subdural haematoma. Cumulative hemispheric area of tissue perfused by sequentially higher levels of CBF was determined at stereotaxic co-ordinate anterior 10.5 mm (relative to bregma). Areas were determined in the contralateral and ipsilateral hemispheres. Data are presented as mean ± S.E.M (n = 6 in each group). There were no significant differences between the vehicle and bosentan treated animals.

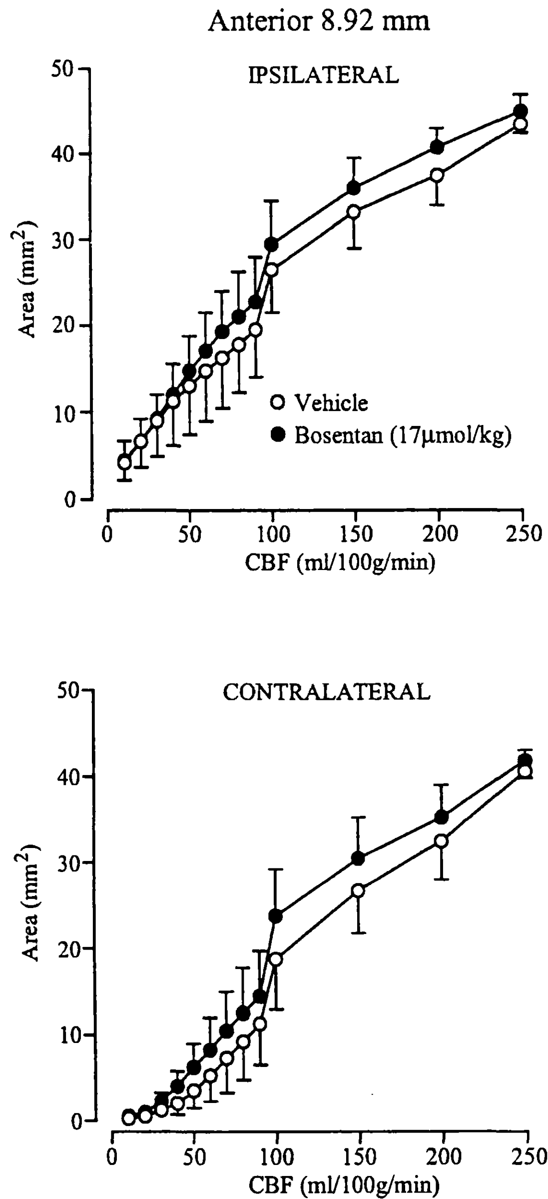


Figure 30 Effects of Bosentan (17 µmol/kg) on cerebral blood flow, determined by frequency distribution analysis, following subdural haematoma. Bosentan (17 µmol/kg) was administered 10 min. prior to the induction of the haematoma and CBF measured 150 min. post-induction of subdural haematoma. Cumulative hemispheric area of tissue perfused by sequentially higher levels of CBF was determined at stereotaxic co-ordinate anterior 8.92 mm (relative to bregma). Areas were determined in the contralateral and ipsilateral hemispheres. Data are presented as mean ± S.E.M (n = 6 in each group). There were no significant differences between the vehicle and bosentan treated animals.

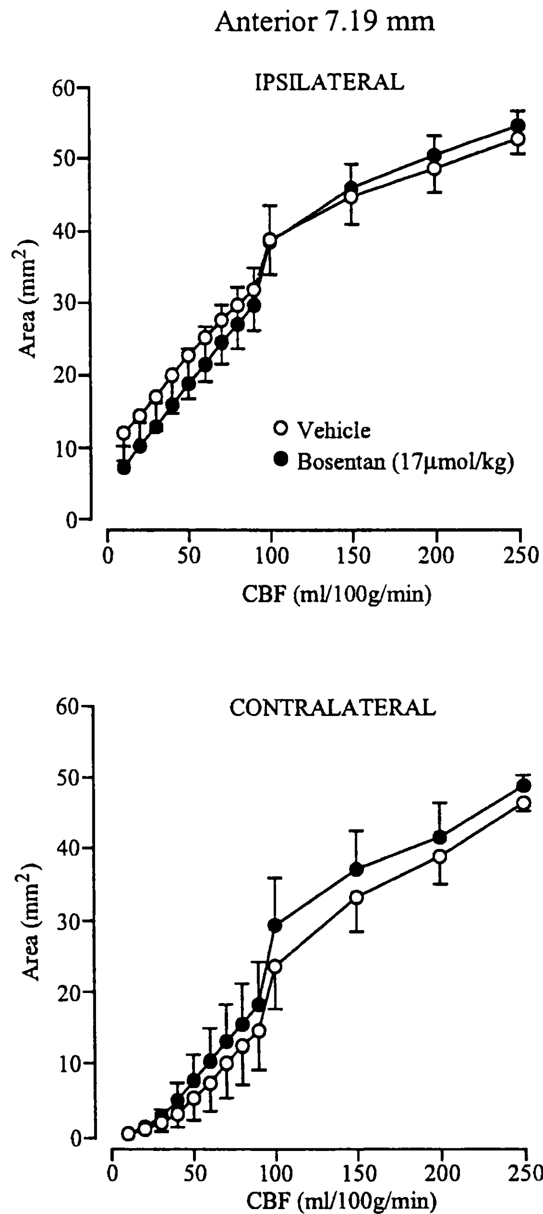


Figure 31 Effects of Bosentan (17 µmol/kg) on cerebral blood flow, determined by frequency distribution analysis, following subdural haematoma. Bosentan (17 µmol/kg) was administered 10 min. prior to the induction of the haematoma and CBF measured 150 min. post-induction of subdural haematoma. Cumulative hemispheric area of tissue perfused by sequentially higher levels of CBF was determined at stereotaxic co-ordinate anterior 7.19 mm (relative to bregma). Areas were determined in the contralateral and ipsilateral hemispheres. Data are presented as mean ± S.E.M (n = 6 in each group). There were no significant differences between the vehicle and bosentan treated animals.

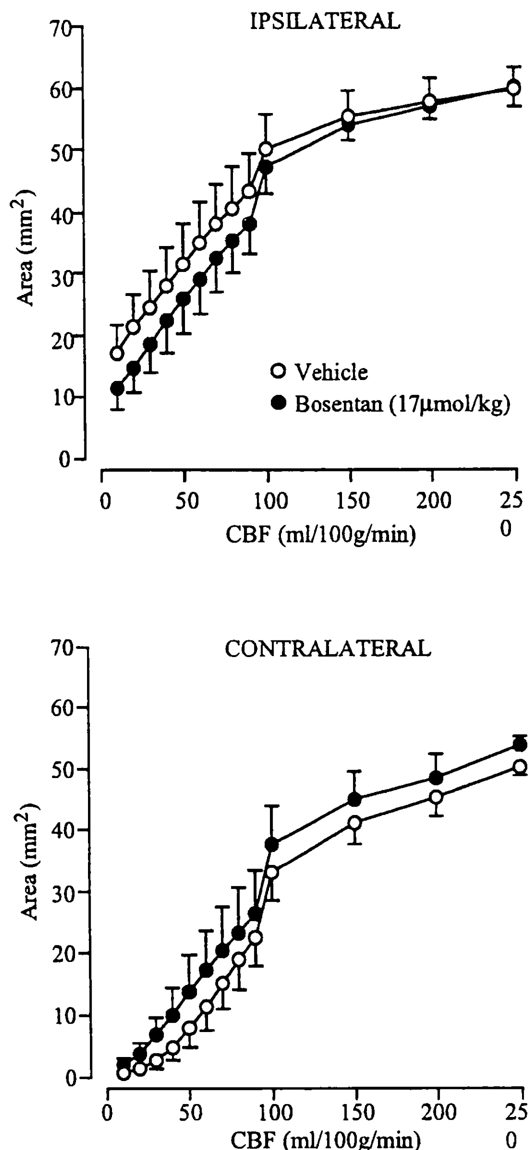


Figure 32 Effects of Bosentan (17 μmol/kg) on cerebral blood flow, determined by frequency distribution analysis, following subdural haematoma. Bosentan (17 μmol/kg) was administered 10 min. prior to the induction of the haematoma and CBF measured 150 min. post-induction of subdural haematoma. Cumulative hemispheric area of tissue perfused by sequentially higher levels of CBF was determined at stereotaxic co-ordinate anterior 6.06 mm (relative to bregma). Areas were determined in the contralateral and ipsilateral hemispheres. Data are presented as mean ± S.E.M (n = 6 in each group). There were no significant differences between the vehicle and bosentan treated animals.

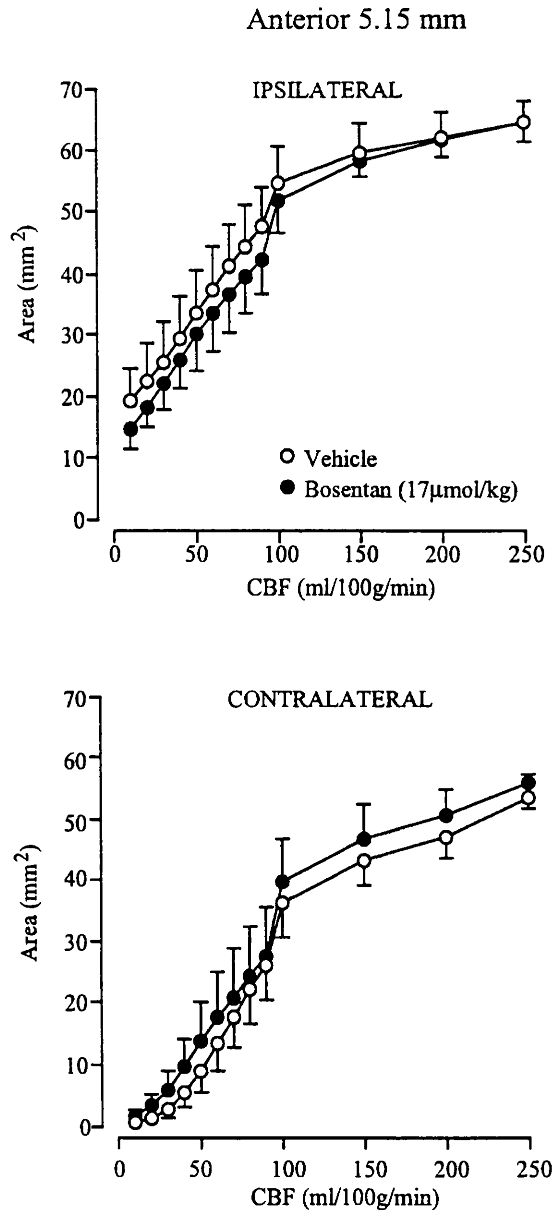
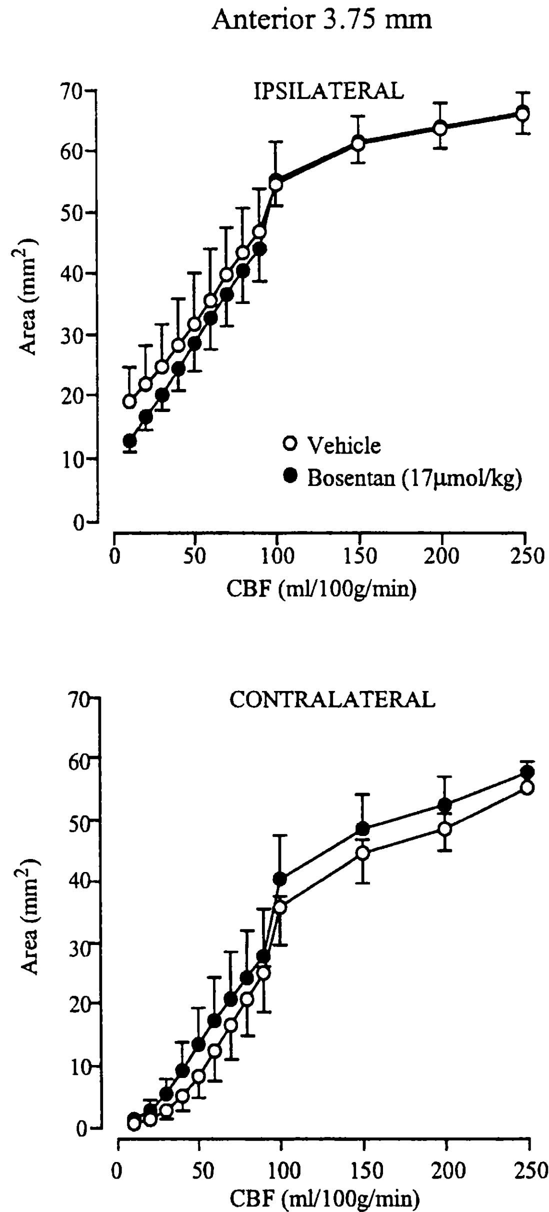


Figure 33 Effects of Bosentan (17 μmol/kg) on cerebral blood flow, determined by frequency distribution analysis, following subdural haematoma. Bosentan (17 μmol/kg) was administered 10 min. prior to the induction of the haematoma and CBF measured 150 min. post-induction of subdural haematoma. Cumulative hemispheric area of tissue perfused by sequentially higher levels of CBF was determined at stereotaxic co-ordinate anterior 5.15 mm (relative to bregma). Areas were determined in the contralateral and ipsilateral hemispheres. Data are presented as mean ± S.E.M (n = 6 in each group). There were no significant differences between the vehicle and bosentan treated animals.



<u>Figure 34</u> Effects of Bosentan (17 µmol/kg) on cerebral blood flow, determined by frequency distribution analysis, following subdural haematoma. Bosentan (17 µmol/kg) was administered 10 min. prior to the induction of the haematoma and CBF measured 150 min. post-induction of subdural haematoma. Cumulative hemispheric area of tissue perfused by sequentially higher levels of CBF was determined at stereotaxic co-ordinate anterior 3.75 mm (relative to bregma). Areas were determined in the contralateral and ipsilateral hemispheres. Data are presented as mean ± S.E.M (n = 6 in each group). There were no significant differences between the vehicle and bosentan treated animals.

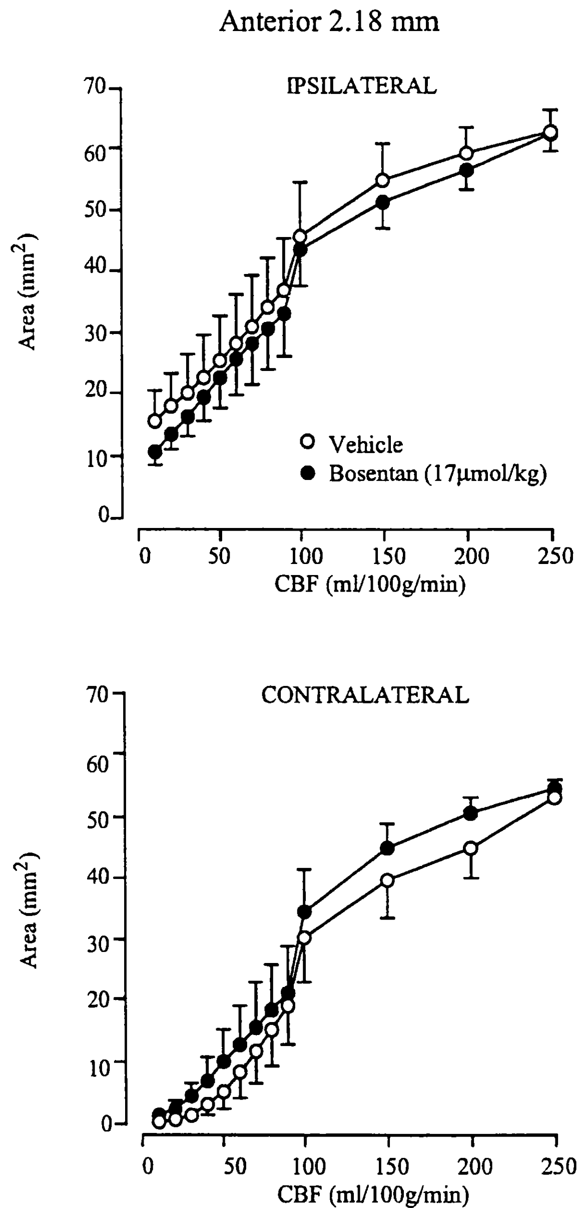


Figure 35 Effects of Bosentan (17 μmol/kg) on cerebral blood flow, determined by frequency distribution analysis, following subdural haematoma. Bosentan (17 μmol/kg) was administered 10 min. prior to the induction of the haematoma and CBF measured 150 min. post-induction of subdural haematoma. Cumulative hemispheric area of tissue perfused by sequentially higher levels of CBF was determined at stereotaxic co-ordinate anterior 2.18 mm (relative to bregma). Areas were determined in the contralateral and ipsilateral hemispheres. Data are presented as mean ± S.E.M (n = 6 in each group). There were no significant differences between the vehicle and bosentan treated animals.

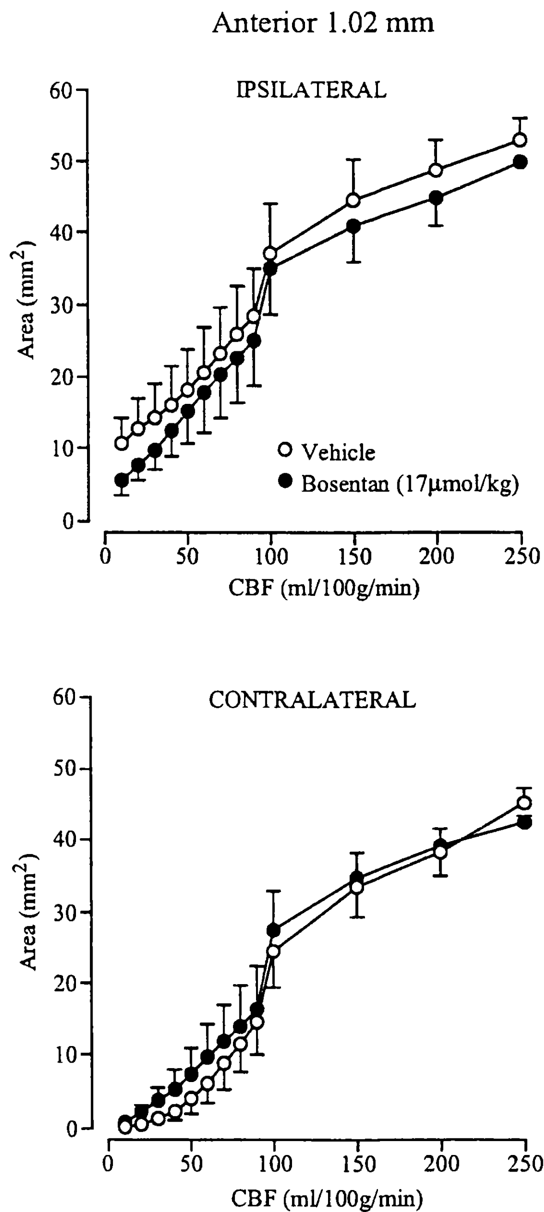


Figure 36 Effects of Bosentan (17 μmol/kg) on cerebral blood flow, determined by frequency distribution analysis, following subdural haematoma. Bosentan (17 μmol/kg) was administered 10 min. prior to the induction of the haematoma and CBF measured 150 min. post-induction of subdural haematoma. Cumulative hemispheric area of tissue perfused by sequentially higher levels of CBF was determined at stereotaxic co-ordinate anterior 1.02 mm (relative to bregma). Areas were determined in the contralateral and ipsilateral hemispheres. Data are presented as mean ± S.E.M (n = 6 in each group). There were no significant differences between the vehicle and bosentan treated animals.

Table 11
Subdural Haematoma in the Rat

	Ipsilateral Hemisphere		Contralateral Hemisphere	
	Vehicle	Bosentan (17μmol/kg)	Vehicle	Bosentan (17μmol/kg)
Cerebellum				
Cerebral Cortex	100 ± 6	114 ± 12	99 ± 7	106 ± 9
Medulla/Pons				
Pontine Gray Matter	109 ± 12	103 ± 12	116 ± 13	103 ± 11
Mesencephalon				
Inferior Colliculus	195 ± 28	193 ± 31	184 ± 17	185 ± 31
Diencephalon				
Medial Geniculate Body	152 ± 39	141 ± 18	214 ± 58	154 ± 36
Sub Thalamic Nucleus	180 ± 34	147 ± 23	196 ± 22	158 ± 24
Lateral Habenular Nucleus	211 ± 26	203 ± 28	256 ± 30	203 ± 26
Hypothalamus	91 ± 14	96 ± 17	87 ± 12	92 ± 15
Mediodorsal Thalamic Nucleus	203 ± 84	122 ± 18	195 ± 71	120 ± 19
Ventrolateral Thalamic Nucleus	153 ± 32	105 ± 17	170 ± 32	120 ± 21
Anterior Thalamic Nucleus	184 ± 54	127 ± 25	214 ± 62	139 ± 30
Telencephalon				
Visual Cortex	39 ± 22	11 ± 2	197 ± 58	114 ± 31
Hippocampus (Molecular Layer)	94 ± 20	105 ± 17	132 ± 28	123 ± 25
Dentate Gyrus	100 ± 22	115 ± 16	139 ± 23	125 ± 20
Auditory Cortex	103 ± 55	123 ± 40	191 ± 46	159 ± 34
Posterior Cingulate Cortex	176 ± 76	50 ± 11	216 ± 70	131 ± 34
Globus Pallidus	87 ± 19	83 ± 12	84 ± 8	74 ± 8
Caudate Nucleus	147 ± 38	119 ± 21	159 ± 34	121 ± 20
Sensory Motor Cortex	24 ± 9	88 ± 42	149 ± 24	143 ± 20
Anterior Cingulate Cortex	326 ± 130	147 ± 32	299 ± 115	142 ± 31
Frontal Cortex	113 ± 53	69 ± 20	181 ± 48	136 ± 31
Parietal Cortex	22 ± 14	6 ± 1	223 ± 75	121 ± 36
Myelinated Fibre Tracts				
Cerebellar White Matter	48 ± 4	49 ± 4	48 ± 4	47 ± 3
Corpus Callosum	36 ± 6	38 ± 8	52 ± 10	42 ± 8
Internal Capsule	50 ± 4	46 ± 6	49 ± 5	47 ± 5
Genu	55 ± 12	44 ± 9	56 ± 11	45 ± 8

Local cerebral blood flow (ml/100g/min) was measured at 150 min following Subdural Haematoma. Data are expressed as mean ± sem (n= 6 in each group). None of the regions examined were significantly diffrent from vehicle.

at the other blood flow thresholds were not significantly altered in the Bosentan treated animals [Figure 37]. There was a marked degree of swelling in the ipsilateral hemisphere of both Bosentan and vehicle treated animals [Figure 38].

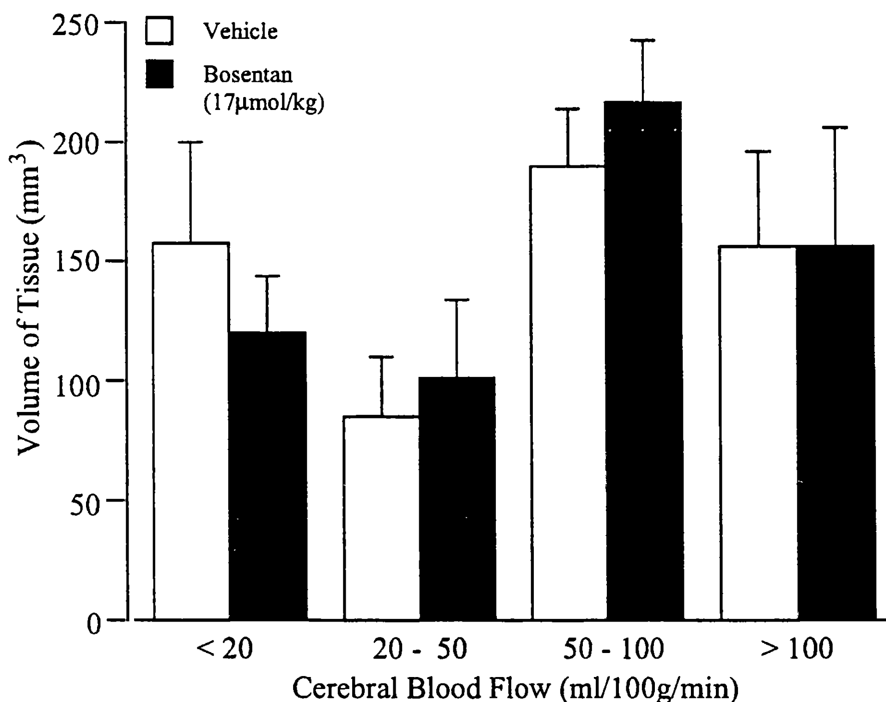


Figure 37 Effect of Bosentan (17 μmol/kg) on the volume of tissue in the ipsilateral hemisphere perfused at pre-determined levels of cerebral blood flow (ml/100g/min) following subdural haematoma in the rat. Bosentan was administered intravenously 10 min. prior to the induction of the haematoma. CBF was determined at 150 min. following the induction of the haematoma. The volume of tissue was determined at CBF levels of <20 ml/100g/min (densely ischaemic), 20-50 ml/100g/min (moderately ischaemic), 50-100 ml/100g/min (oligaemic) and >100 ml/100g/min (normal tissue). Data are expressed as mean ± S.E.M (n = 6 in each group). There were no significant differences between the vehicle and Bosentan treated animals.

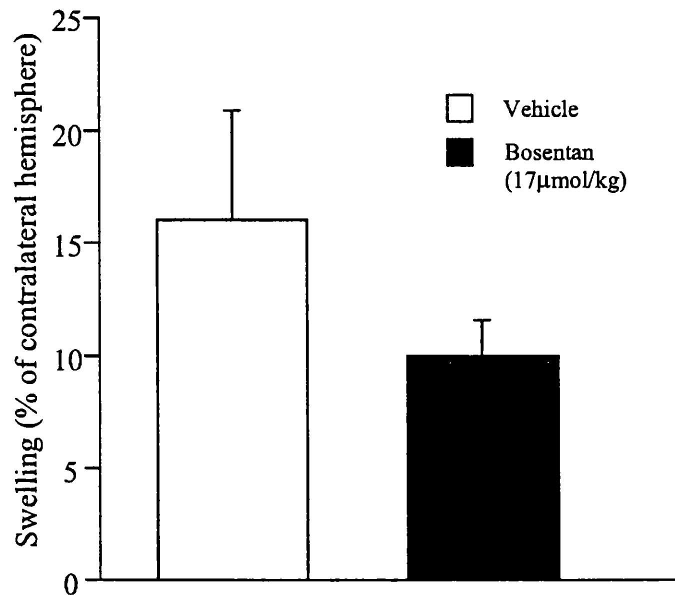


Figure 38 Effect of Bosentan (17 μmol/kg) on the swelling of the ipsilateral hemisphere following subdural haematoma in the rat. Bosentan was administered intravenously 10 min. prior to the induction of the haematoma. Swelling was calculated from the difference between the volumes of the ipsilateral and contralateral hemispheres. Data are expressed as mean ± S.E.M percent of the contralateral hemisphere (n = 6 in each group). There were no significant differences between the vehicle and Bosentan treated animals.

3.3.4 Alterations in Pial Arteriolar Calibre following Permanent MCA Occlusion in the Cat

At the outset of the study the mean arterial blood pressure was 86 ± 2 mmHg, arterial pH was 7.42 ± 0.02, arterial pCO_2 was 31 ± 2 mmHg and arterial pO_2 201 ± 5 mmHg and were maintained within these ranges for the duration of experiment.

Post MCA occlusion alterations in vascular diameter

The cranial window in these investigations exposed the ectosylvian, suprasylvian and parasagittal gyri. The blood supply of the ectosylvian and suprasylvian gyri is normally derived from the middle cerebral artery whereas the supply for the parasagittal gyrus is derived primarily from the anterior cerebral artery. In the period immediately following MCA occlusion all vessels in all the regions of the preparation appeared to very labile. The vessels overlying the ectosylvian and suprasylvian gyri appeared to undergo an initial marked dilatation of approximately 50% of pre occlusion vessel calibre. In 4 cats, all arterioles on the ectosylvian and suprasylvian gyri displayed sustained marked dilatation. In 2 cats, there were widespread zones of sustained marked vasoconstriction (reductions in arteriolar calibre of more than 50% from pre-occlusion levels) in both ectosylvian and suprasylvian gyri. In 1 cat, there was a circumscribed zone of constriction confined to the ectosylvian gyrus which was labile over time; elsewhere in the ectosylvian and suprasylvian gyri there were marked dilatations of the pial arterioles. The onset of vasoconstriction varied considerably among the vessels. In a few, it was well developed by 10 min. after MCA occlusion; in others it began to develop 20 min. after MCA occlusion. Once vasoconstriction had developed, it was generally sustained. Of the 25 vessels in total whose calibre was followed sequentially for 30 min. post-occlusion, 15 displayed marked sustained dilatation; 10 displayed marked sustained vasoconstriction. The frequency of constriction/dilatation reflects anatomical bias in the selection of vessels for sequential assessment. The cerebral arterioles

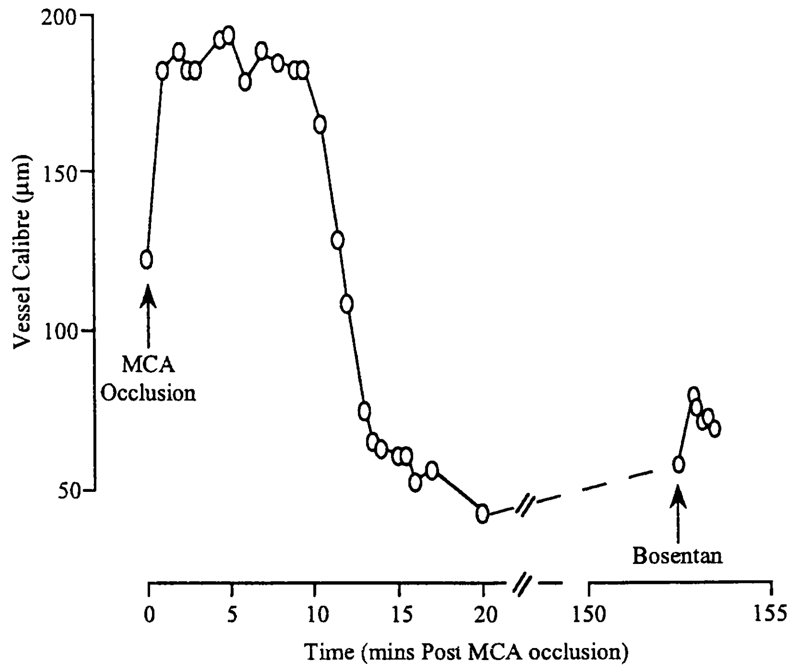
overlying the parasagittal gyrus appeared to undergo a marked vasodilatation that was sustained for the remainder of the experimental period.

Perivascular microapplication of Bosentan and CSF on arterioles following permanent MCA occlusion

Bosentan (30 μM) was applied into the perivascular space surrounding the post occlusion dilated and constricted arterioles overlying the ectosylvian and suprasylvian gyri. Bosentan (30 μM) effected a marked increase in post ischaemic pial arteriolar calibre of all vessels, constricted and dilated (29.3 ± 5.5% from baseline ; n = 51). The magnitude of the responses after microapplication of Bosentan (30 μM) was numerically greater in the vessels displaying post occlusion constriction (68.2 ± 16.5 % from baseline; n = 13 ; P< 0.01 for the comparison with CSF (pH 7.2) on constricted vessels) [Figure 39] compared to vessels displaying post occlusion dilatation (16 ± 8.9 % from baseline; n = 38; P < 0.01 for the comparison with CSF (pH 7.2) on dilated vessels) [Figure 40]. The <u>diameter</u> of the post occlusion dilated vessels increased from 175 ± 10.8 μm to 202.2 ± 12.9 μm following the microapplication of Bosentan. The <u>diameter</u> of the post occlusion constricted vessels increased from 97.2 ± 14 μm to 153 ± 20.7 μm following the microapplication of Bosentan. The responses of the dilated arterioles to the microapplication of Bosentan increased as the experimental period progressed [Figure 41; Figure 42].

The perivascular microapplication of CSF (pH 7.2) on post occlusion constricted and dilated vessels (overlying the ectosylvian and suprasylvian gyri) resulted in small consistent vasoconstrictions of the arterioles (-8.6 ± 1% from baseline ; n = 52) . The magnitude of the CSF (pH 7.2) effect was similar on the dilated (-8.7 ± 0.9 % from baseline; n = 44) and constricted vessels (-7.9 ± 4.7 % from baseline; n = 8) overlying the suprasylvian and ectosylvian gyri [Figures 39 & 40]. There appeared to be little variation in these responses over the experimental period [Figure 41; Figure 42].

Bosentan (30 μM) and CSF (pH 7.2) were also applied to the vessels overlying the parasagittal gyrus. These vessels appeared to undergo dilatation following middle



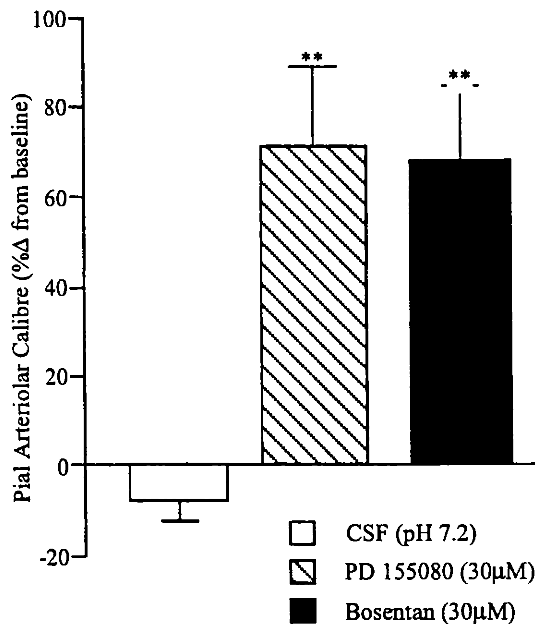
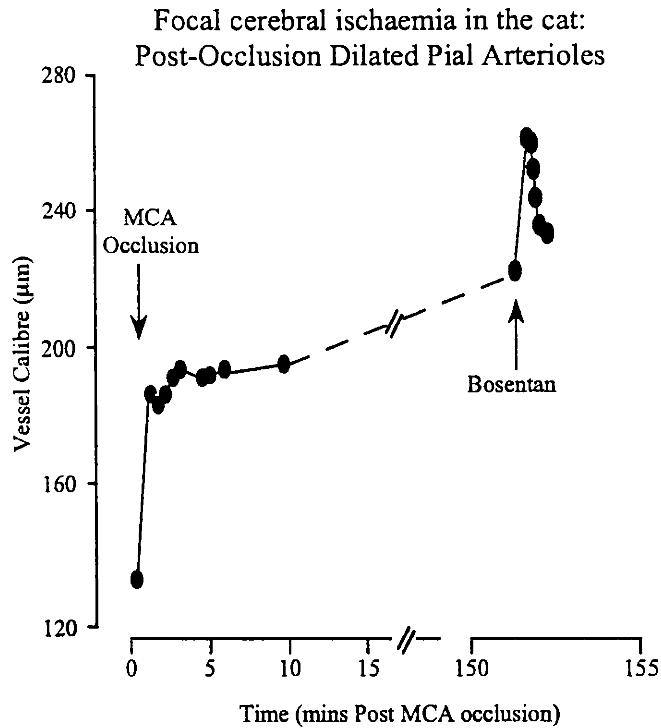


Figure 39 (Top) Vasomotor responses of a post-occlusion constricted pial arteriole to the microapplication of Bosentan (30 μM). Perivascular microapplication of Bosentan elicited a transient increase in the calibre of the arteriole.

(Bottom) Alterations in the calibre of post-occlusion constricted pial arterioles. Microapplications of CSF, Bosentan (30 μM) and PD155080 (30 μM) were carried 30-210 min. following MCA occlusion. Data are expressed as mean ± S.E.M percent change from pre-injection baseline (n = 8-13 in each group). *P<0.01 for the comparison between CSF and Bosentan or PD155080



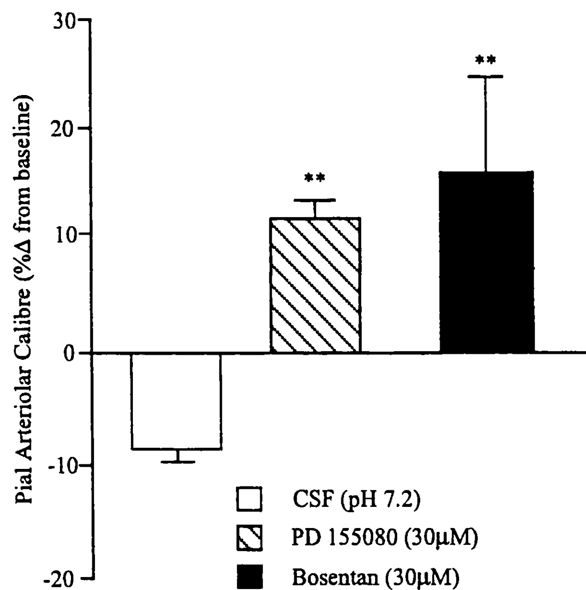


Figure 40 (Top) Vasomotor responses of a post-occlusion dilated pial arteriole to the microapplication of Bosentan (30 μM). Perivascular microapplication of bosentan elicited a transient increase in the calibre of the arteriole.

(Bottom) Alterations in the calibre of post-occlusion dilated pial arterioles. Microapplications of CSF, Bosentan (30 μM) and PD155080 (30 μM) were carried out 30-210 min. following MCA occlusion. Data are expressed as mean ± S.E.M percent change from pre-injection baseline (n = 36-44 in each group). *P<0.01 for the comparison between CSF and Bosentan or PD155080

cerebral artery occlusion. CSF (pH 7.2) elicited a small vasoconstrictive response (-7.1 ± 3% from baseline; n = 5) and the microapplication of Bosentan (30 μM) had minimal effect on vessel calibre (-2.1 ± 2.1% from baseline ; n = 7).

Perivascular Microapplication of PD155080 and CSF on arterioles following permanent MCA Occlusion

The perivascular microapplication of standard CSF resulted in small consistent reductions in arteriolar calibre in the post-occlusion period (-8.6 ± 1.0%; n=52). The magnitude of the reduction in calibre produced by CSF was similar in vessels displaying post-ischaemic constriction and post-ischaemic dilatation. The magnitude of the reduction in calibre produced by CSF was relatively constant throughout the post-ischaemic period evaluated [Figure 41; Figure 42].

The perivascular microapplication of PD155080 in standard CSF resulted in increases in arteriolar calibre in the post-occlusion period (21.2 ± 4.7%; n=44, p<0.001 compared to CSF). The magnitude of the increase in calibre after microapplication of PD155080 was numerically greater in vessels displaying post-ischaemic constriction compared to vessels displaying post-ischaemic dilatation [Figure 39; Figure 40]. The responses of arterioles to the microapplication to PD155080 increased as the post-occlusion time period increased [Figure 41; Figure 42].

PD155080 (30 μM) and CSF (pH 7.2) were also applied to the vessels overlying the parasagittal gyrus. These vessels appeared to undergo dilatation following middle cerebral artery occlusion. CSF (pH 7.2) elicited a small vasoconstrictive response (-7.1 ± 3% from baseline; n = 5) and the microapplication of PD155080 (30μM) had minimal effect on vessel calibre (-1.5 ± 3.1% from baseline ; n = 5).

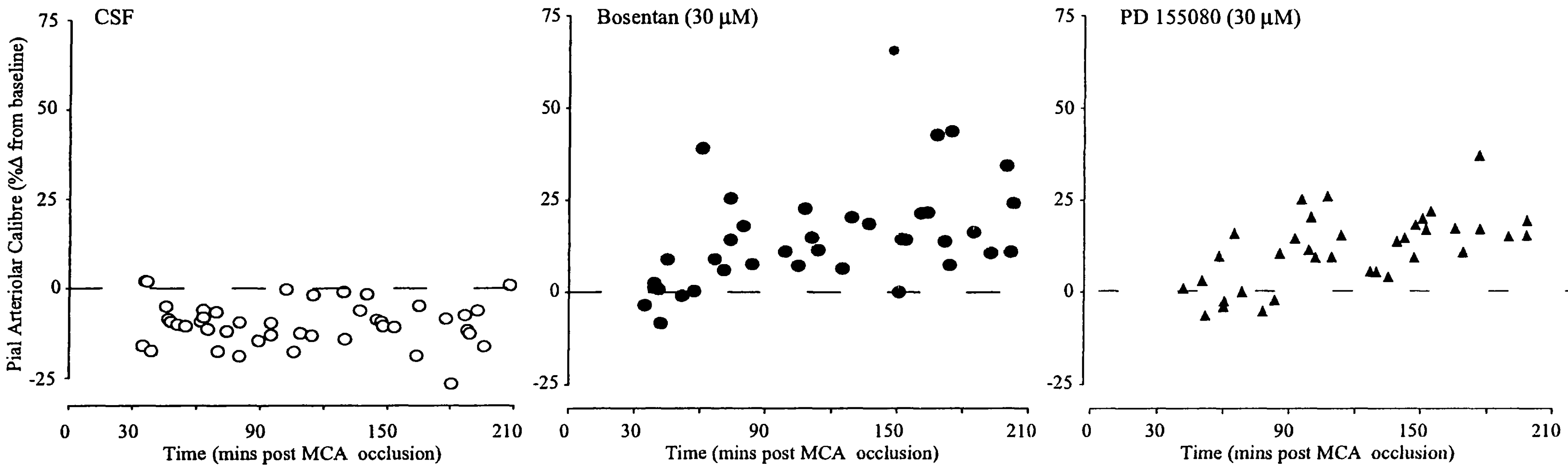


Figure 41 Responses of individual pial arterioles to CSF, Bosentan (30 μM) and PD155080 (30 μM) at varying times after middle cerebral artery occlusion. All of the arterioles had displayed sustained vasodilatation after middle cerebral artery occlusion. Each point represents the response of a single pial arteriole. The middle cerebral artery was occluded at time zero. Data are expressed as percent change from pre-injection baseline.

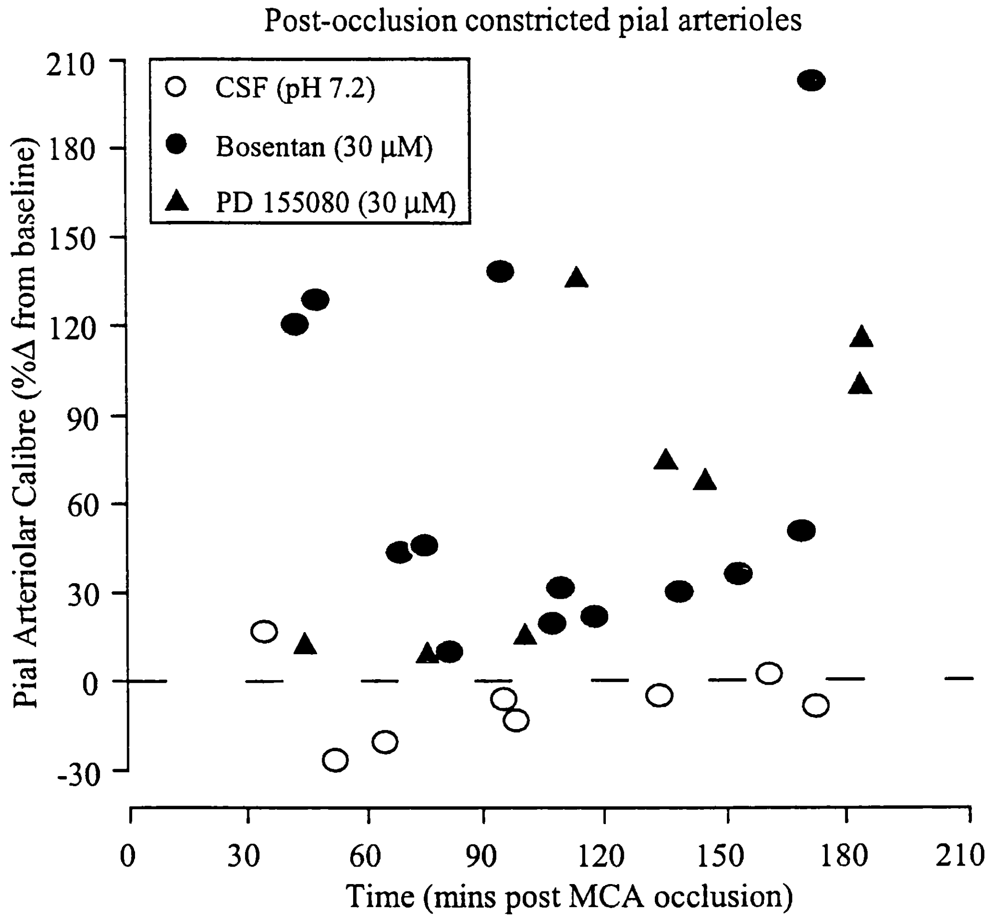


Figure 42 Responses of individual pial arterioles to CSF, Bosentan (30 μM) and PD155080 (30 μM) at varying times after middle cerebral artery occlusion. All of arterioles had displayed transient vasodilatation followed by sustained vasoconstriction after middle cerebral artery occlusion. Each point represents the response of a single pial arteriole. The middle cerebral artery was occluded at time zero.Data are expressed as percent change from pre-injection baseline.

CHAPTER 4

DISCUSSION

The potent vasoconstrictor actions of the endothelins has led to the speculation of its potential role in health and disease. The identification of endothelin receptor subtypes and the subsequent development of endothelin receptor antagonist has greatly facilitated the understanding of the role of the endothelins. The role of the endothelins in the regulation of vascular tone was the corner stone of research, however, recent evidence has demonstrated that the endothelins may have a role as spasmogens, growth factors and as neuromodulators.

Since the primary publication describing the structure and functions of the endothelins, there have been an rapid developments describing the role of the endothelins in physiology and pathophysiology and the therapeutic potential of the endothelin receptor antagonists.

In the rabbit basilar artery in vitro, the ET-1 induced contractions were resistant to antagonism by either Bosentan or BQ-123. In contrast, the ET-3 induced contractions were markedly antagonised by BQ-123. The activation of endothelin ET_B receptors demonstrated no significant contractile effect. These results suggested that the ET-1 induced contractions were mediated by an 'atypical' endothelin ET_A receptor. The in situ investigations in the cat demonstrated the presence of vasoconstrictor endothelin ET_A receptors and dilator endothelin ET_B receptors. The results in this thesis indicated that in addition to species differences in the profiles of endothelin receptors, there may be variations in the distribution of endothelin receptors across the cerebrovascular tree (from conduit vessels to cerebral resistance arterioles). The differences in the potency of endothelin receptors antagonist against ET-1/ET-3 in the rabbit basilar artery suggests that the current classification of endothelin receptors may not adequately describe the responses of the endothelin receptor agonists.

The investigations to evaluate the efficacy of Bosentan demonstrated that Bosentan antagonised the vasoconstriction induced by exogenous ET-1 in feline cerebral resistance arterioles. The ET-1 induced vasoconstriction was significantly attenuated following the intravenous administration of Bosentan. The intravenous administration of ET_A receptor antagonist PD155080 attenuated the vasoconstrictive effect of exogenous ET-1. In contrast, the intravenous administration of the peptide

antagonist PD145065 had no significant effect on the ET-1 induced vasoconstriction in feline cerebral arterioles. The present thesis contrasted the ability of the non-peptide endothelin receptor antagonists and inability of the peptide endothelin receptor antagonists to gain access to the abluminal surface of cerebral resistance arterioles following systemic administration. The investigation of the pathophysiologic role of the endothelins requires the use of non-peptide endothelin receptor antagonists.

Post-ischaemic cerebral hypoperfusion was observed following severe transient global cerebral ischaemia. The intravenous administration of Bosentan failed to alter post-ischaemic hypoperfusion assessed by hydrogen clearance and Iodoantipyrine autoradiography. No hypoperfusion was observed following moderate transient global cerebral ischaemia. The inability of Bosentan to alleviate post-ischaemic hypoperfusion following severe transient global cerebral ischaemia indicates that the endothelins may play a minor role in the maintenance of hypoperfusion. The reductions in hippocampal CA1 neuronal damage by endothelin receptor antagonists indicates that the endothelins may play a role in the development of neuronal damage.

Cerebrocortical regions underlying the subdural haematoma displayed a profound reduction in cerebral blood flow. There was an increase in cerebral oedema in the cerebral cortex underlying the haematoma. Bosentan failed to alter cerebral blood flow or brain swelling following subdural haematoma in the rat. The failure of an endothelin receptor antagonist to demonstrate efficacy suggests that the endothelins may have a minor role in the reductions in cerebral blood flow associated with a subdural haematoma. However, the small sample size prevents us from conclusively excluding a role for the endothelins in the mediation of the neuronal damage. The proven efficacy of the endothelin receptor antagonist in experimental subarachnoid haemorrhage suggests that the endothelins may have a delayed role following haemorrhagic injury in the brain.

The non-peptide endothelin ET_A/ET_B receptor antagonist Bosentan increased the post-ischaemic vascular diameter of cerebral resistance arterioles following permanent occlusion of the MCA in the cat. The magnitude of the increases in post-

occlusion vascular diameter with the non-peptide endothelin ET_A receptor antagonist PD155080 was similar to Bosentan. These investigations demonstrated the existence of an increased endothelin mediated tone in cerebral resistance vessels in the ischaemic penumbra. The demonstration of these effects provides a basis for examining the therapeutic role of the endothelin receptor antagonists in the regulation of penumbral cerebral perfusion and ischaemic neuronal damage following a focal cerebral ischaemia.

4.1 ENDOTHELIN RECEPTORS AND ENDOTHELIN RECEPTOR ANTAGONISTS IN THE CEREBROVASCULATURE

4.1.1 Endothelin Receptors

In contrast to the vast literature on the characterisation of endothelin receptors in the peripheral vasculature, there is a paucity of information concerning the endothelin receptors in the cerebrovasculature. A number of investigators have characterised the endothelin receptors in the major cerebral arteries (spinal, basilar, middle cerebral) in different species [Adner et al., 1993; Kitazono et al., 1993; Salom et al., 1993; Feger et al., 1994; Willette et al., 1994; Schilling et al., 1995]. Investigations in the peripheral vasculature indicate that the endothelin ET_A receptor is the predominant receptor mediating ET-1 induced vasoconstrictions [Warner et al., 1993; Masaki et al., 1994; Rubanyi & Polokoff, 1994]. The role of endothelin ET_B receptors is more controversial with endothelin ET_B receptor mediated vasodilatation and vasoconstriction reported in different vascular beds [Clozel et al., 1992; Sumner et al., 1992; Shetty et al., 1993].

In vitro investigations

The present investigations examined the effects of endothelin receptor activation in the rabbit basilar artery. The receptor characterisation of the endothelin receptor subtypes in the middle cerebral arteries, basilar arteries and the spinal arteries from the goat, guinea pig, rat and dog have indicated that the contractile responses to the endothelins are mediated by the endothelin ET_A receptors (similar to the effects described in most peripheral vessels) (see Table 12). Endothelin ET_B receptor mediated vasodilatation has been described in *in vitro* investigations [Rubanyi & Polokoff, 1994].

In the present investigations the combined endothelin ET_A/ET_B receptor antagonist Bosentan and the endothelin ET_A receptor selective antagonist BQ-123 demonstrated rightward shifts of the endothelin-1 concentration response curves. These responses indicate that the antagonism elicited by these receptors is competitive

Table 12

Responses of isolated cerebral arteries to endothelin receptor agonists

Species	Vessel	Agonist	EC_{50}	Response	Receptors	References
Rat	Intracerebral arterioles	ET-1	0.7 nM	vasoconstriction		Edwards & Trizna, 1990
		ET-1	4.8 pM - 1.9 nM	vasoconstriction		Ogura et al., 1991
	Basilar artery	ET-1	7.9 nM	vasoconstriction	ET_A	Feger et al., 1994
		ET-3	170 nM	vasoconstriction		Feger et al., 1994
		ET-3	1.5 nM	vasodilatation	ET_B	Schilling et al., 1995
Guinea Pig	MCA	ET-1	3 nM	vasoconstriction	ET_A	Adner et al., 1993
Rabbit	Basilar artery	ET-1	5.8 nM	vasoconstriction		Feger et al., 1994
		ET-1	11.5 nM	vasoconstriction	ET_A	present thesis
		ET-3	194 nM	vasoconstriction		present thesis
Feline	MCA	ET-1	0.17 nM	vasoconstriction		Jansen et al., 1989
		ET-1	3 nM	vasoconstriction		Kauser et al., 1990
Canine	Anterior spinal artery	ET-1	2 nM	vasoconstriction	'atypical' ET_A	Willette et al., 1994
	Basilar artery	ET-1	0.4 nM	vasoconstriction		Saito et al., 1991

Table 12

		ET-1	0.6 nM	vasoconstriction		Tanoi et al., 1991
		ET-1	4 nM	vasoconstriction	ET_A	Willette et al., 1994
		ET-3	26.5 nM	vasoconstriction		Saito et al., 1991
	PCA	ET-1	0.7 nM	vasoconstriction		Tanoi et al., 1991
	MCA	ET-1	9 nM	vasoconstriction		Garcia et al., 1991
Goat	MCA	ET-1	2 nM	vasoconstriction		Vila et al., 1990
		ET-1	3 nM	vasoconstriction		Diequez et al., 1992
		ET-1	0.3 nM	vasoconstriction	ET_A	Salom et al., 1992
		ET-1	0.5 nM	vasoconstriction		Salom et al., 1992
		ET-3	0.1 µM	vasoconstriction		Salom et al., 1992
Porcine	Basilar artery	ET-1	1.7 nM	vasoconstriction		Fukuda et al., 1991
	ACA	ET-1	2 nM	vasoconstriction		Taga et al., 1990
		ET-1	1.9 nM	vasoconstriction		Fukuda et al., 1991
Bovine	MCA	ET-1	10 nM	vasoconstriction		Ferrer et al., 1992
Human	Meningeal artery	ET-1	24.5 nM	vasoconstriction		Hardebo et al., 1989
	Superficial temporal	ET-1	16.9 nM	vasoconstriction		Hardebo et al., 1989
		ET-1	0.8 nM	vasoconstriction		Papadopoulos et al., 1990
	MCA	ET-1	1 nM	vasoconstriction		Martin de Aguilera et al., 1990

in nature although the pA_2 values, 5.1 and 5.3 respectively, suggested that the contractile responses of ET-1 were resistant to these antagonists. The pA_2 values for Bosentan and BQ-123 in the present investigations are at variance with the values obtained in the peripheral vasculature (100 fold) and in cerebral vessels [Ihara et al., 1992; Feger et al., 1994; Rubanyi & Polokoff, 1994]. The characterisation of endothelin receptors in cerebral vessels in other species (goat, rat, guinea pig, dog) have indicated that the vasoconstrictor actions of the endothelins are mediated by the endothelin ET_A receptors (see Table 13). The potency of the endothelin ET_A receptor antagonists used in the investigations are comparable to the potencies obtained in the peripheral vasculature. The rabbit basilar artery was 100 fold less sensitive to ET-3 mediated vasoconstrictions compared to ET-1, indicating an endothelin ET_A receptor mediated contractile response. BQ-123 was more potent in antagonising the contractile actions of ET-3 than ET-1. The increased potency of endothelin receptor antagonist in attenuating the responses of ET-3 compared to ET-1 have been observed in the rat vas deferens [Eglezos et al., 1993]. In the rabbit basilar artery, the endothelin ET_B receptor agonist BQ-3020 elicited contractions of reduced magnitude and sensitivity compared to ET-1. The results obtained in the present studies are in contrast to the observations in a rabbit model of subarachnoid haemorrhage where the combined endothelin ET_A/ET_B receptor antagonist PD145065 demonstrated greater efficacy in attenuating vasospasm than the endothelin ET_A antagonist BQ-610 [Zuccarello et al., 1994].

The segments of rabbit basilar artery used in the investigations were denuded of the endothelial layer. This was confirmed by the absence of any significant relaxation following the addition of 10 µM acetylcholine. The presence of endothelin ET_B receptors in the rabbit basilar artery were examined using the selective receptor agonist BQ-3020. The magnitude of the contractile response to BQ-3020 was substantially less than the contractile responses elicited by either ET-1 or ET-3. These observations indicated that in the rabbit basilar artery the endothelin mediated constrictions were mediated primarily by the endothelin ET_A receptor. However, the differential potencies of BQ-123 in antagonising ET-1 or ET-3 suggests that the receptor may be a subtype of the endothelin ET_A receptor [Bax & Saxena, 1994].

Table 13

Table 13

Endothelin receptors in peripheral vascular and non-vascular tissues

Species/tissue	Receptor	Response	Reference
Rat			
Aorta	ET_A	vasoconstriction	Karaki et al., 1993
	ET_B	vasodilatation	Karaki et al., 1993
	'atypical' ET_A	vasoconstriction	Sumner et al., 1992
Renal artery	ET_A/ET_B	vasoconstriction	Wellings et al., 1994; Cristol et al., 1993
Coronary artery	ET_A/ET_B	vasoconstriction	
Pulmonary artery	ET_A		
Pulmonary arterioles	ET_B	vasoconstriction	McCulloch & MacLean , 1995
	'atypical' ET_A	vasoconstriction	McCulloch & MacLean , 1995
Mesenteric	ET_A/ET_B		
Atrium	'atypical' ET_B	contraction	Panek et al., 1992
Myocardium	ET_B	positive inotropic effect	Takanishi et al., 1991; Kasai et al., 1994
Trachea	ET_A/ET_B	contraction	Clozel & Gray, 1995
Stomach fundus	'atypical' ET_B	contraction	Warner et al., 1993
	ET_B	contraction	Gray et al., 1995
	ET_A/ET_B	relaxation	Gray et al., 1995
Vas deferens	'atypical' ET_A	contraction	Eglezos et al., 1993; Warner et al., 1993

Table 13

Guinea pig			
Aorta	ET_A	vasoconstriction	Hay et al., 1993
Pulmonary artery	ET_A	vasoconstriction	Cardell et al., 1992
Mesenteric arterioles	ET_A	vasoconstriction	Berthiaume et al., 1995
Iliac artery	ET_A	vasoconstriction	Schoeffter et al., 1993
Trachea	ET_B	contraction	Cardell et al., 1992; Takai et al., 1992
Bronchus	ET_B	contraction	Hay et al., 1993
Gastric Smooth Muscle	ET_A	contraction	Chijiiwa et al., 1995
Rabbit			
Pulmonary artery	ET_A/ET_B	vasoconstriction	La Douceur et al., 1993; White et al., 1993; Fukuroda et al., 1994
Carotid artery	ET_A	vasoconstriction	Moreland et al., 1992; White et al., 1993
Iliac artery	ET_A	vasoconstriction	Moreland et al., 1992
Jugular vein	ET_B	vasoconstriction	White et al., 1993
Saphenous vein	ET_{B1}	vasodilatation	Douglas et al., 1995
	ET_{B2}	vasoconstriction	Douglas et al., 1995; Moreland et al., 1992
	'atypical' ET_A	vasoconstriction	Douglas et al., 1995
Feline			
Hind limb arterioles	ET_A	vasoconstriction	Ekelund et al., 1993; Ekelund et al., 1995
	ET_B	vasodilatation	Ekelund et al., 1995

Table 13

Canine			
Coronary artery	ET_B	vasoconstriction	Teerlink et al., 1994
Hepatic artery	ET_A	vasoconstriction	Faro et al., 1995
Saphenous vein	ET_B	vasoconstriction	Moreland et al., 1992
Iliac artery	ET_A	vasoconstriction	Moreland et al., 1992
Porcine			
Pulmonary artery	'atypical' ET_B	vasoconstriction	Sudwarjo et al., 1993; Fukuroda et al., 1992
	ET_B	vasodilatation	Saeki et al., 1991; Ihara et al., 1992
Pulmonary vein	'atypical' ET_B	vasoconstriction	Sudwarjo et al., 1993; Fukuroda et al., 1992
Coronary artery	'atypical' ET_B	constriction	Schoeffter et al., 1993
	ET_A/ET_B	vasoconstriction	Fukuroda et al., 1992; Balwierczak et al., 1993
Coronary vein	'atypical' ET_A	vasoconstriction	Fukuroda et al., 1992
Renal artery	ET_A	vasoconstriction	
Human			
Aorta	ET_A	vasoconstriction	Maguire et al., 1995; Davenport et al., 1995; Maguire & Davenport, 1994
Pulmonary artery	ET_A	vasoconstriction	Maguire et al., 1995; Davenport et al., 1995; Maguire & Davenport, 1994; Godfraind, 1993

Table 13

Pulmonary arterioles	'atypical' ET_A	vasoconstriction	McCulloch & MacLean, 1995
	ET_B	vasoconstriction	McCulloch & MacLean, 1995
Coronary artery	ET_A	vasoconstriction	Dagassan et al., 1995; Davenport et al., 1995; Maguire & Davenport, 1994
	'atypical' ET_A	vasoconstriction	Godfraind et al., 1993
Saphenous vein	ET_A	vasoconstriction	Maguire et al., 1995; Davenport et al., 1995; Maguire & Davenport, 1994
	'atypical' ET_A	vasoconstriction	Bax et al., 1993
Brachial artery	ET_A/ET_B	vasoconstriction	Haynes et al., 1995; Haynes & Webb, 1994
Internal mammary artery	ET_A/ET_B	vasoconstriction	Seo et al., 1994
	ET_A	vasoconstriction	Maguire et al., 1995; Davenport et al., 1995
Umbilical vein	'atypical' ET_A	vasoconstriction	Bodelsson et al., 1993
Renal artery	ET_A	vasoconstriction	Maguire et al., 1995; Davenport et al., 1995; Maguire & Davenport, 1994
Renal vein	ET_A	vasoconstriction	Maguire et al., 1995; Davenport et al., 1995
Omental artery	ET_A	vasoconstriction	Reizebos et al., 1994
Omental vein	'atypical' ET_A	vasoconstriction	Riezebos et al., 1994
Bronchus	ET_B	contraction	Hay et al., 1993
Myometrium	ET_A	contraction	Heluy et al., 1995

The results obtained in the rabbit basilar artery indicate that the contractile responses effected by ET-1 and ET-3 may be mediated by an endothelin receptor that does not conform to current classification of endothelin ET_A/ET_B receptors [Bax & Saxena, 1994]. An ET-3 preferring receptor, putatively called the endothelin ET_C receptor has been described in the amphibian [Karne et al., 1993]. There has been a report of a receptor specific for ET-3 in bovine cultured endothelial cells although the significance of such a receptor is yet to be identified [Morita et al., 1992]. Unlike the endothelin ET_A and endothelin ET_B receptors, the gene cloning the endothelin ET_C receptor has not been isolated in mammalian species. Recently, there have been reports that the prolonged incubation of vascular segments *in vitro* with the endothelin receptor antagonists results in the degradation of the antagonists [Wu Wong et al., 1995].

In the periphery, tissues where the rank order of potencies of the endothelin receptor agonists has suggested the presence of an endothelin ET_A receptor (ET-1 more potent than ET-3) [Rubanyi & Polokoff, 1994]. The antagonism of the ET-3 mediated constrictions were greater than the ET-1 mediated constrictions at these receptors with BQ-123 in the rat aorta and jugular vein [Sumner et al., 1992]. In the cerebral circulation, similar responses have been observed in the goat middle cerebral arteries where BQ-123 was more potent at antagonising the sarafotoxin 6b mediated constrictions than the ET-1 mediated vasoconstrictions [Salom et al., 1995]. The rank order of potencies of the sarafotoxins and the endothelins at the endothelin receptors indicates that sarafotoxin 6b is less potent at the endothelin ET_A receptors than ET-1 [Rubanyi & Polokoff, 1994]. The existence of atypical responses in tissues previously thought to contain endothelin ET_B receptors has also been demonstrated (see Table 13).

There are no clear explanations for the difference in the potency of BQ-123 in antagonising ET-1 or ET-3 in the rabbit basilar artery. The potency of BQ-123 as antagonist for the ET-1 mediated responses has been demonstrated in the rat basilar artery [Feger et al., 1994]. The investigations in the rat basilar artery indicated the presence of constrictor ET_A receptors and vasodilator ET_B receptors [Feger et al.,

1994; Schilling et al., 1995]. In the present investigations the endothelin-1 constriction appears to be mediated by ET_A receptors but the reduced potency of the BQ-123 suggests that there may be subtypes of endothelin receptors in the rabbit basilar artery. The hypotheses that have been explored include the possibility that the binding of ET-3 to the endothelin ET_A receptor may be more reversible than the binding of ET-1 to same receptor and, thus, BQ-123 is more potent at antagonising the ET-3 mediated constrictions than the ET-1 mediated effects [Battistini et al., 1994; Wu Wong et al., 1994 &1995]. Although one cannot discount the possibility of there being differences in the binding kinetics of the endothelin receptor agonists and antagonists, there is the possibility that the ET-1 and ET-3 may be exerting their effects via subtypes of the endothelin ET_A receptor and there may be differences in the affinities of the putative receptor subtypes for ET-1, ET-3 and BQ-123.

In vivo investigations

The cerebral resistance arterioles play the major role in the regulation of cerebral blood flow to the brain and there have been few investigations examining the effects of endothelin receptor activation in cerebral resistance arterioles *in vivo*. The present investigations have demonstrated that endothelin ET_A receptors mediate vasoconstriction while endothelin ET_B receptors mediate vasodilatation in feline small cerebral resistance arterioles *in vivo*. The failure of the intracarotid infusion of BQ-3020 to alter cerebral arteriolar calibre indicates that the endothelin ET_B receptors may not be readily accessible by intraluminal endothelin receptor agonists which are peptide analogues.

The role of endogenous endothelin in the regulation of vascular tone in peripheral and cerebral blood vessels has been suggested [Yoshimoto et al., 1990; Haynes & Webb, 1994]. In feline peripheral blood vessels, an absence of basal endothelin mediated tone has been demonstrated [Ekelund et al., 1993; Ekelund et al., 1994; Ekelund et al., 1995]. In the current investigations the adventitial microapplication of the endothelin ET_A receptor antagonist BQ-123 had minimal effect of the calibre of the pial arterioles. The perivascular microapplication of other

endothelin receptor antagonists, e.g. Bosentan, PD145065, PD155080, have demonstrated similar effects indicating that under the present experimental conditions there is minimal endothelin mediated tone in the cerebral resistance arterioles. These observations contrast with the reports of Yoshimoto et al. (1990) which suggest that there is a basal production of endothelin by cerebrovascular endothelial cells in culture, and those of Haynes and Webb (1994) which indicate the presence of basal endothelin mediated tone in human forearm vessels. The adventitial co-application of ET-1 with increasing concentrations of BQ-123 demonstrates a dose dependent attenuation of the ET-1 induced vasoconstriction. The estimated IC_{50} for BQ-123 determined from these experiments is 0.7 μM and this is comparable to the values obtained for this antagonist in other tissues [Ihara et al., 1991]. In feline skeletal muscle, the constriction of resistance arterioles is mediated by endothelin ET_A receptors while endothelin-1 mediated vasodilatation is mediated by endothelin ETB receptors [Ekelund et al., 1993; Ekelund et al., 1994]. In cerebral resistance arterioles it appears that the vasoconstrictor effect of ET-1 is mediated primarily via endothelin ET_A receptors. The ability of BQ-123 to attenuate the effects of exogenous ET-1 in cerebral resistance arterioles is similar to the responses obtained with the combined endothelin ET_A/ET_B receptor antagonists Bosentan and PD145065 and endothelin ET_A receptor antagonist PD155080.

Previous investigations of Armstead et al. (1989) in the piglet and Faraci (1989) in the rat indicated the existence of endothelin mediated vasodilatation in cerebral resistance arterioles. However, the absence of receptor selective agonist and antagonist prevented the characterisation of the endothelin receptor subtypes. In the present investigations, the adventitial microapplication of BQ-3020 effected a dose dependent vasodilatation of cerebral resistance arterioles *in vivo*. These observations are in agreement with reports indicating that endothelin ET_B receptors mediate vasodilatation in the rat basilar artery *in vivo* and *in vitro* [Kitazono et al., 1993; Feger et al., 1994; Schilling et. al., 1995]. Preliminary investigations have demonstrated that the BQ-3020 mediated vasodilatation can be attenuated by the combined endothelin ET_A/ET_B receptor antagonist Bosentan (T.R. Patel unpublished observations)

suggesting that the endothelin ET_{B1} receptor subtype may mediate vasodilatation in feline cerebral arterioles [Masaki et al., 1994]. The cranial window technique used in the present investigations allows us to examine cerebral resistance arterioles (40-350 μm) under normal physiologic and neurogenic control. This *in situ* preparation facilitates the observation of vasodilator responses of cerebral arterioles without pre-constriction. Previous investigations have demonstrated that the responses of cerebral resistance arterioles *in vivo*, to vasodilator or vasoconstrictor agents, may be dependent on the pre-injection calibre of arterioles *in vivo* [Kuschinsky & Wahl, 1975; Harper & MacKenzie, 1977; McCulloch & Edvinsson, 1980]. The present investigations have demonstrated that cerebral arterioles less than 100 μm elicited a greater vasodilator response following the adventitial application of BQ-3020. In the present study we have been unable to demonstrate a similar correlation following the adventitial application of ET-1 (10 nM). The absence of a correlation with ET-1 may be due to the small number of arterioles examined. We have re-examined the responses of cerebral arterioles to ET-1 from the present study and those conducted previously and can demonstrate a significant correlation between the pre-injection calibre of arterioles and response to adventitial ET-1 [Figure 43]. The responses of arterioles to the microapplication of CSF was not dependent on the calibre of the arterioles [Figure 43].

Intracarotid infusion of BQ-3020 failed to demonstrate a significant alteration in arteriolar calibre of cerebral resistance vessels. A distinguishing feature of the cerebral circulation is the blood-brain barrier. The blood-brain barrier can prevent the access of molecules, e.g. peptides, to the adventitial surface of cerebral arterioles [Ermisch et al., 1993]. Endothelin receptor agonists or antagonists targeted at the cerebrovasculature would have to gain access to the abluminal surface of cerebral arterioles in order to exert their effects. BQ-3020 with its peptide structure would not be expected to penetrate the blood-brain barrier and gain access to the abluminal surface of cerebral vessels. Previous investigations using intraluminal administration of ET-1 have demonstrated alterations in cerebral blood flow and cerebral blood volume [Kobari et al., 1994a; Willette et al., 1990]. The investigations of Willette and colleagues (1990a, 1990b) demonstrated alterations in cerebral blood flow following

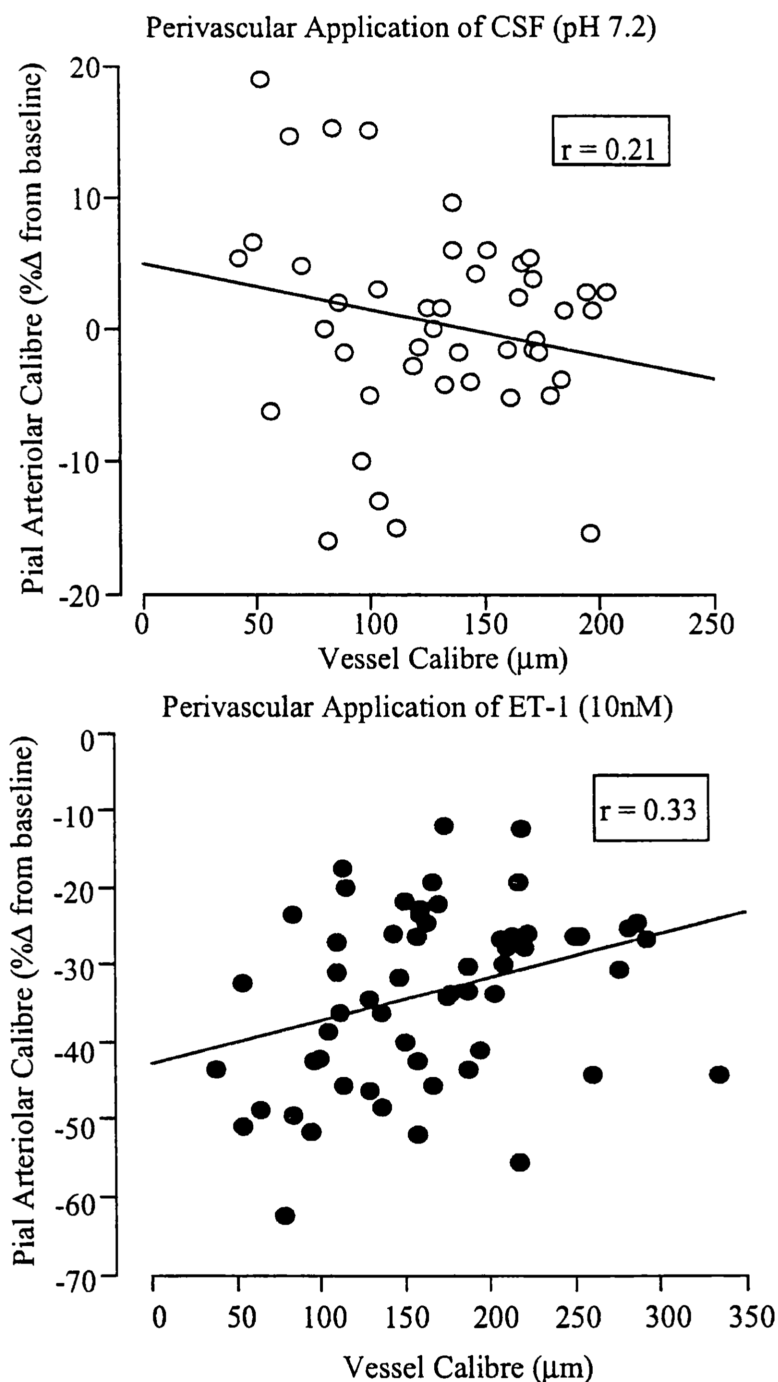


Figure 43 The dependence of the pial arteriolar responses elicited by CSF (pH 7.2) and endothelin-1 (10 nM) on the calibre of arterioles examined. Pial arteriolar diameter was determined prior to the adventitial microapplication of CSF or endothelin-1. The arteriolar responses are expressed as the percent change from pre-injection baseline. There is no significant association between the arteriolar responses to CSF and the pre-injection calibre. *Post hoc* analysis of these responses have demonstrated significant association between the arteriolar response to endothelin-1 and pre-injection calibre (r^2 =0.11; P <0.01). (see text for references).

the intravenous or intracerebroventricular administration of endothelin-1. The effects of low doses of endothelin administered elicited a hypotensive response and the increases in CBF observed would be related to a autoregulatory response. The intracerebroventricular administration of ET-1 may have the effect of inducing a local ischaemia at the site of action and may explain the reductions in CBF. The intraluminal infusions of ET-1 induces systemic hypertension, and hypertension *per se* produces alterations in cerebral arteriolar calibre as part of the cerebral autoregulatory response. ET-1 can also activate endothelin ET_A and endothelin ET_B receptors and for these reasons it was not examined in the present investigations. The failure of intracarotid infusions, in contrast to the adventitial application, of BQ-3020 to alter arteriolar calibre indicates that the endothelin ET_B receptors may be located on the abluminal surface of cerebral resistance arterioles. In the present investigations, assuming a carotid blood flow of 5 ml/min., we estimate that the intravascular concentration of BQ-3020 (with these infusion rates) would be in the range 0.1-100 nM. The adventitial microapplication of these concentrations of BQ-3020 elicit a dilatation of cerebral resistance arterioles. The failure of the intracarotid infusion of BQ-3020 to alter pial arteriolar calibre is at variance with the observations of Kobari et al. (1994b). The differences in the observations could be the result of the different methodological approaches employed in the two investigations. Kobari and colleagues (1994b) have used alterations in cerebral blood volume as an indicator of cerebral vasodilatation. Cerebral blood volume is a measure of cerebral capacitance (a combination of arterial, arteriolar, capillary and venous diameters) and alterations in cerebral capacitance will not necessarily reflect increases in cerebral blood flow. In the present investigations, BQ-3020 was administered as a continuous infusion while Kobari and colleagues administered the agents as a bolus [Kobari et al., 1994b]. The alterations in cerebral blood volume reported may be the result of an injection artefact [Kobari et al., 1994b]. Evidence from *in situ* investigations have indicated the susceptibility of endothelin receptors to desensitisation [Hollenberg et al., 1993]. The initial intracarotid infusions of low doses of BQ-3020 could have resulted in the desensitisation of endothelin ET_B receptors in the cerebral arterioles. The infusion of

500 pmol/min. of BQ-3020 resulted in a transient reduction in MABP indicating that the endothelin ET_B receptors in the peripheral vasculature were activated.

In the rat basilar artery, endothelium dependent vasodilatation has been demonstrated following activation of endothelin ET_B receptors [Feger et al., 1994; Schilling et al., 1995]. The vasodilatation in the rat basilar artery is mediated by nitric oxide [Feger et al., 1994; Schilling et al., 1995]. The mediation of the dilator response following the activation of endothelin ET_B receptors is unknown but a functional link to either nitric oxide or prostacyclin has been suggested [Hirata et al., 1993]. The present investigations do not discount the existence of endothelin ET_B receptors on the luminal surface of endothelial cells or on cerebrovascular smooth muscle since northern blot analysis and functional studies on human blood vessels have indicated the existence of receptors without a functional link to either nitric oxide or prostacyclin [Luscher, 1993; Seo et al., 1994].

The cat has been used for investigating the role of the endothelins in the pathophysiology of focal cerebral ischaemia. The present investigations indicate that the location of the endothelin ET_B receptors is on the abluminal surface of cerebral resistance arterioles and that the endothelin ET_B receptors mediate vasodilatation. The dilatation of cerebral resistance arterioles would result in an increase in cerebral blood flow. The ability of pharmacologic agents to increase cerebral blood flow would have beneficial effects in conditions of impaired cerebral blood flow e.g. stroke. In contrast to the description of endothelin ET_B receptor mediated vasoconstriction in peripheral tissues, similar responses have not been observed in cerebral arteries or arterioles *in vitro* or *in situ* [Table 12; Table 14]. The present investigations indicate a vasoconstrictor action for endothelin ET_A receptors in feline cerebral resistance arterioles. The present investigations suggest that antagonists selective for the endothelin ET_A receptors may be of greater utility than the combined endothelin ET_A/ET_B receptor antagonists in cerebrovascular investigations.

Endothelin Receptors in the Central Nervous System

Table 14

In vivo responses of cerebral arteries and arterioles to endothelin receptor agonists

Species	Vessel	Agonist	EC_{50}	Response	Receptors	References
Rat	Basilar artery	ET-1	30 nM	vasoconstriction		Faraci et al., 1989
		ET-1	6 nM	vasoconstriction		Murray et al., 1992
	Pial arterioles	ET-1	10 nM	vasoconstriction		Faraci et al., 1989
	Microvessels	ET-1	30 nM	vasoconstriction		Willette & Sauermelch, 1990
Rabbit	Basilar artery	ET-1	20 nM	vasoconstriction	ET_A/ET_B	Foley et al., 1994; Zuccarello et al., 1994
Feline	Basilar artery	ET-1	30 nM	vasoconstriction		Mima et al., 1989
	Pial vessels	ET-1	10 nM	vasoconstriction	ET_A	Robinson & McCulloch, 1990; present thesis
		BQ-3020	30 nM	vasodilatation	ET_B	present thesis
	Microvessels	ET-1	3 nM	vasodilatation	ET_B	Kobari et al., 1994a; Kobari et al., 1994b
Canine	Basilar artery	ET-1	30 nM	vasoconstriction		Mima et al., 1989
Goat	MCA	ET-1	0.6 nM	vasoconstriction		Salom et al., 1991
Porcine	Pial arterioles	ET-1	1.2 nM	vasoconstriction		Armstead et al., 1989

The presence of endothelin-1 and endothelin-3 has been demonstrated in the brains of a variety of species [Gulati, 1995]. Endothelin immunoreactivity has been demonstrated in neurones and glial cells in numerous brain regions including the paraventricular nuclei, supraoptic nuclei, hypothalamus, pituitary gland, hippocampus and the spinal cord neurones [Matsumoto et al., 1989; Yoshizawa et al., 1990; Shinmi et al., 1989a; Shinmi et al., 1989b; Giaid et al., 1989]. The regional distribution of endothelin-1 mRNA and immunoreactivity has indicated high concentrations in the cerebral cortices, hippocampus, hypothalamus cerebellar regions and the spinal cord in the rat and human brain [Matsumoto et al., 1989; Yoshizawa et al., 1990; Shinmi et al., 1989a; Shinmi et al., 1989b; Giaid et al., 1989; Lee et al., 1990]. The co-localisation of endothelin converting enzyme with the endothelin-1 mRNA and immunoreactivity in human and rat brain suggests a potential role of the endothelins as neuromodulatory agents [Gulati, 1995].

Endothelin receptor binding sites have been described in glial cells (astrocytes) and neurones (e.g. dorsal root ganglia) [Niwa et al., 1992; Jones et al., 1989; Giaid et al., 1989; MacCumber et al., 1990; Greenberg et al., 1992; Koseki et al., 1989]. Binding studies with radiolabelled endothelin-1 have indicated the presence of binding sites in the cerebellum, cerebral cortex, dentate gyrus in the hippocampus and in nuclei in the brainstem [Jones et al., 1989; Kurihara et al., 1990; Nambi et al., 1990]. The indications are that the predominant receptor subtype in the cerebral cortex, cerebellum and astrocytes is the endothelin ET_B receptor [MacCumber et al., 1990; Nambi et al., 1990; Vigne et al., 1991]. The presence of ET mRNA has been described in glial cells (astrocytes) and neurones (e.g. dorsal root ganglia) [Niwa et al., 1992; Jones et al., 1989; Giaid et al., 1989; MacCumber et al., 1990; Greenberg et al., 1992; Koseki et al., 1989]. Early investigations provided evidence for ET mRNA in cells of the hypothalamus, striatum and also the spinal cord [Giaid et al., 1989; Lee et al., 1990]. Secretion of endothelin-1 by astrocytes has been reported and can be stimulated by thrombin and platelet derived growth factor (PDGF) [MacCumber et al., 1990; Lysko et al., 1991; Fuxe et al., 1992; Marsault et al., 1990; Ehrenreich et al., 1991; Federici et al., 1995; Levin et al., 1992]. The role of endothelins in the CNS is unclear,

but, stimulation of immediate early gene expression (c-Fos) and nerve growth factor in astrocytes indicates that these peptides may play a role in the regeneration of nerve cells following a cerebrovascular injury [Ladenheim et al., 1993].

A number of investigators have performed functional studies to examine the effects of exogenous endothelins in the brain. Injections of endothelin-1 into the cisterna magna or microinjections into discrete brainstem nuclei in the rat have elicited marked alterations in cardiovascular parameters with systemic hypertension and bradycardia being the most prominent [Siren & Feuerstein, 1989; Ferguson & Smith, 1990; Macrae et al., 1991]. The measurement of cerebral blood flow in the rat brainstem following an intracisternal injection of endothelin-1 demonstrated a marked reduction in cerebral blood flow to pathological levels [Macrae et al., 1991]. Other investigations have indicated that the endothelins may play a role as neuromodulators in the locomotor system or in the release of pituitary hormones [Gross et al., 1994; Gross et al., 1993]. The intracerebroventricular injection of endothelin-1 elicited behavioural effects such as barrel rolling and oculo-clonic seizures [Gross et al., 1994; Gross et al., 1993] and are associated with alterations in cerebral blood flow and cerebral glucose utilisation in peri-ventricular regions and areas distal to the site of injection [Gross et al., 1994; Gross et al., 1993]. These observations suggest a role for the endothelins as neuromodulators or neuropeptides [Gross et al., 1994; Berrino et al., 1994]. The alterations in CBF and glucose utilisation were attenuated by the calcium channel and endothelin antagonists. However, the effects of endothelin receptor antagonists *per se* on neuronal function has not been examined.

The demonstration of high density of endothelin immunoreactivity and binding sites in the pituitary and hypothalamus suggests a role for the endothelins in the modulation of pituitary hormone release [Koseki et al., 1989; Nambi et al., 1990; Niwa et al., 1991]. The intravenous and intracerebroventricular administration of ET-1 and ET-3 has been demonstrated to increase the plasma concentrations of adrenocorticotrophic hormone and inhibit the release of prolactin. There has been speculation on a role for ET-1 in stimulating the release of growth hormone, lutenising hormone, follicle stimulating hormone and gonadotropin releasing hormone [Samson

et al., 1990]. Recent investigations have demonstrated the ability of ET-1 to increase plasma concentrations of arginine vasopressin and atrial natriuretic peptide. The mechanism of action of ET-1 in eliciting these increases is unclear.

The role of the endothelins in the central control of cardiovascular and respiratory function has been an area of much interest from a therapeutic standpoint [Ferguson et al., 1995]. The intracisternal administration of ET-1 elicits a rise in mean arterial blood pressure and increased heart rate [Ferguson et al., 1995; Gulati et al., 1995]. These observations have been combined with electrophysiologic studies which have demonstrated that the centrally administered ET-1 increases the neuronal firing in areas of the medulla that are specifically linked to the control of cardiovascular function [Ferguson et al., 1995; Gulati et al., 1995]. The conclusions drawn from these investigations suggested a role for the endothelins as neuromodulator. The direct administration of the endothelins into the cisterna magna resulted in brain stem ischaemia and the reflex response increased the blood pressure and heart rate [Macrae et al., 1991]. Similarly, direct injection of ET-1 into discrete cardiovascular control nuclei in the brain would result in local ischaemia. The failure of a brain penetrating endothelin receptor antagonist to alter cerebral blood flow or blood pressure in the rat suggests that the control of basal CBF or blood pressure may not have a major endothelin component [McAuley et al., 1994]

Concluding Remarks

The *in vitro* and *in vivo* investigations conducted in the course of the present thesis have indicated that in the rabbit basilar artery and the feline cerebral resistance arterioles, the endothelin receptor mediated constrictions are mediated by endothelin ET_A receptors. The observations are in broad agreement from the studies carried out in peripheral vascular tissues where a similar receptor profile was observed. Recent data from receptor binding investigations have demonstrated the presence of endothelin ET_A receptors on the medial layer of the anterior cerebral artery in the rat [De Oliveira et al., 1995]. However, the species differences in the distribution of receptors in the

various vascular beds precludes the direct extrapolation of information from one species to the other.

The studies carried out in the course of the present thesis, have utilised three different species, namely the rat, the cat and the rabbit. The investigations and results obtained are limited by the techniques used to classify the receptors in the species used. A critical appraisal of the *in vitro* and *in situ* techniques with respect to the quality of information that can be obtained from these techniques has been discussed in the following section.

A criticism of the attempts to characterise the receptors in cerebral vessels is the use of different species and different techniques to evaluate the functions of endothelin receptors in the cerebrovasculature. The studies conducted in the larger cerebral arteries, e.g. the middle cerebral artery, and basilar artery in the rat have indicated that the vasoconstriction is mediated by endothelin ET_A receptors while the existence of endothelin ET_B receptors has been demonstrated on the abluminal surface and on the vascular endothelium (Table 13). The receptors mediating endothelin constriction and dilatation are broadly similar in peripheral tissue and the cerebral blood vessels across the species. Recent evidence has indicated that there may be a population of receptors that do not conform to the normal classification of endothelin receptors (see Table 13). The receptors mediating vasoconstriction in the rabbit basilar artery may indeed belong to this group.

The identification of the endothelin receptor subtypes in the cerebrovasculature could have been approached using receptor binding techniques as has been used by some authors [Pierre & Davenport, 1995; Davenport & Maguire, 1994]. However, identification of the endothelin receptors in the cerebrovasculature would be limited by the size of vessels and the functional significance of endothelin receptors in the vessels. The existence of endothelin receptor binding sites is not a clear indication that the those receptors would be functionally linked to the signal transduction mechanisms [Luscher, 1993; Seo et al., 1994].

The application of exogenous endothelin on cerebral vessels can decrease cerebral blood flow to levels that induce ischaemic pathology [Macrae et al., 1993;

Fuxe et al., 1992]. These observations have fuelled an increasing interest in the role of endothelins in cerebrovascular pathology. Increases in plasma and tissue endothelin levels have been observed following subarachnoid haemorrhage and ischaemic stroke in experimental models and humans [Ziv et al., 1992; Suzuki et al., 1992; Duverger et al., 1992, Barone et al., 1994; Bian et al., 1994]. The recent development of endothelin receptor selective antagonists has seen these compounds investigated in a variety of cerebrovascular pathologies [Clozel and Watanabe, 1992; Clozel et al., 1993; Nirei et al., 1993; McAuley et al., 1994; Ohlstein et al., 1994; Zuccarello et al., 1994]. The stimulation of endothelin ET_B receptors, either by receptor agonism or selective antagonism of endothelin ET_A receptors, would be expected to increase cerebral blood flow. The availability of brain penetrating endothelin ET_A receptor antagonists would be of considerable value in manipulating the vasoconstrictor action of endothelins in ischaemic injury [Feuerstein et al., 1994; Ohlstein et al., 1994].

Critical appraisal of in vitro and in situ techniques used for investigating responses of cerebral vessels

An understanding of the responses of cerebral vessels to vasodilator and vasoconstrictor agents is a fundamental part of cerebrovascular research. There have been descriptions of the existence of perivascular nerves containing a range of neurotransmitters and neuropeptides [see section 1.1.3]. A role for the vascular endothelium in the regulation of cerebrovascular tone has also been described [see section 1.1.3]. A complex interaction between the transmitters and peptides may exist under physiologic or pathophysiologic conditions. The use of *in situ* and *in vivo* techniques is essential for the identification of the role of the substances in the regulation of cerebrovascular tone under normal and pathophysiologic conditions.

The vessels of the cerebral circulation differ in their morphologic characteristics from the vessels in the peripheral circulation. The inflow arteries such as the basilar artery contain approximately 8-10 smooth muscle cell layers. The posterior cerebral artery, middle cerebral artery and the anterior cerebral artery contain 5-6 smooth muscle cells on the circumference. The pial arteries and arterioles on the cortical surface contain 2-3 vascular smooth muscle layers on the circumference while the penetrating arterioles contain 1-2 vascular smooth muscle cell layers. The vascular endothelium in the cerebral circulation comprises the blood-brain barrier [see section 1.1.2 for description].

The differences in the morphology of the cerebral vessels has led to the development of two main techniques for cerebrovascular investigations. *In vitro* techniques utilise isolated cerebral blood vessels. The *in situ* techniques are used to examine the cerebral blood vessels in their normal physiologic milieu.

In vitro techniques

The *in vitro* techniques used to examine the vasomotor responses of cerebral vessels to the endothelins have used ring segments of cerebral vessels, helical strips and cannulation of cerebral vessels [e.g. Saito et al., 1989; Saito et al., 1991; Feger et

al., 1994; Salom et al., 1995; Schilling et al., 1995]. The ring segments are the most commonly used preparation. The cerebral vessels are divided into rings and metal probes are inserted through the lumen of the segment. One of the probes is secured to the organ bath while the other probe is attached to a tension transducer to measure isometric force. Investigations have been performed using helical strips of the cerebral vessels and the responses of the strips are measured using tension transducers. The use of helical strips for examining the vasomotor responses has a disadvantage because the width and pitch of the strip will affect the responsiveness. More recent developments have seen the use of cannulations of cerebral vessels to measure vasomotor responses [Saito et al., 1991]. The two ends of the dissected vessel are cannulated with glass cannulae and the vessel perfused at a constant pressure.

There are a number of advantages of the use of *in vitro* techniques for investigating vasomotor responses of cerebral vessels. The use of *in vivo* techniques invariably involves the use of anaesthetic agents, such as the barbiturates and halothane, and these agents have been shown to affect vasomotor reactivity [Fitch et al., 1983; Bonvento et al., 1994]. The examination of cerebral vessels *in vitro* allows the examination of vasomotor responses in the absence of anaesthetic effects. The use of organ baths permits a greater control of the surrounding milieu than would be achieved in the *in vivo* situation. The effect of hypoxia, hypocapnia, hypercapnia and pH on the vasomotor responses of cerebral vessels has been well documented [see Edvinsson et al., 1993 for details; Kuschinsky et al., 1972; McCulloch et al., 1982]. The *in vitro* techniques also allow the examination of the vasomotor effects of cerebral vessels isolated from a number of species. The use of cerebral vessels such as the middle cerebral artery or basilar artery from different species facilitates the comparisons between the species for that vessel. Additionally there is evidence to indicate the existence of multiple receptors and receptor subtypes on cerebral vessels [Edvinsson & Owman, 1974; Feger et al., 1994]. The use of receptor antagonists can aid the isolation of receptor subtype under investigation, e.g. ß-adrenoceptor antagonists for examining the effects of α-adrenoceptor activation. The interactions

between the neurotransmitters and their receptors present in cerebral vessels can be effectively examined using *in vitro* techniques.

There is considerable evidence indicating the inability of peptide molecules to gain access to the cerebrovasculature following their systemic administration [Ermisch et al., 1993]. The blood-brain barrier prevents access of intravascularly administered endothelin to the cerebrovascular smooth muscle [Willette & Sauermelch, 1990a & 1990b; Kobari et al., 1994a]. The use of isolated vascular segments *in vitro* has facilitated the identification of the role of the endothelins in the mediation of the vasomotor responses. The administration of peptide molecules like endothelin to the organ bath facilitates the access of the peptide molecule to the cerebrovascular smooth muscle and constrict the cerebral vessel [See Table 12]. The significance of the vascular endothelium in the regulation of vascular responses has added another dimension to the understanding of the responses of cerebral vessels. The use of *in vitro* techniques have facilitated the examination of the effect of neurotransmitters and neuropeptides in the presence and absence of the vascular endothelium. The vascular endothelium releases dilator substances (nitric oxide, prostacyclin) and transmitters such as acetylcholine release nitric oxide from the vascular endothelium and facilitate vasodilatation [see section 1.1.3]. However, the examination of vasorelaxation *in vitro* requires the preconstriction of the vessels and there is a risk of interaction between the pharmacological agents used for constricting the vessel and the use of the transmitter for the examination of vasodilatation.

The use of *in vitro* techniques for the examination of the vasomotor responses of cerebral vessels is limited by the size of vessel that can be examined. The size of the vessel can also determine the *in vitro* technique used. The larger cerebral vessels such as the middle cerebral artery and the basilar artery can be examined using ring segments or helical strips, although the species (rat, feline, canine, bovine, primate or human) from which the artery was dissected will affect the decision since the size of the major cerebral arteries will vary. The development of wire and pressure myographs for the measurement of vascular responses has facilitated the examination of the vascular responses of cerebral arteries and resistance arterioles (diameter 200-500 μm)

[Kauser et al., 1990]. The wire myograph measures the isometric force developed by the vascular segment . In the pressure myograph a length of the vessel is perfused at a pressure that is representative of the perfusion pressure *in vivo* [Kauser et al., 1990]. The pressure myograph allows the examination of the vascular responses following the intraluminal or extraluminal administration of the neurotransmitter or neuropeptide e.g. endothelin [Saito et al., 1991].

A major issue in cerebrovascular research is the contribution of the resistance arterioles in the regulation of cerebral blood flow compared to the contribution of the major cerebral arteries [Stromberg & Fox, 1972]. The development of *in vitro* methodology for the measurement of vasomotor responses has enhanced the understanding of the role of the resistance arterioles and the major cerebral arteries in the regulation of cerebral blood flow.

In situ techniques

The *in situ* techniques developed have concentrated on the measurement of the vascular responses of the cerebral resistance arterioles overlying the cortical surface (pial arterioles). The morphology of the pial arterioles compared to the major cerebral arteries and intraparenchymal arterioles has been discussed at the beginning of this section. The pial arterioles are innervated by sympathetic and parasympathetic nerves and the neurotransmitters and neuropeptides released can regulate the vasomotor responses of the arterioles [see section 1.1.3]. There is evidence that the cortical pial arterioles may be a transitional zone for the blood-brain barrier as indicated by the permeability to small neutral amino acids [Blasberg et al., 1983]. The permeability characteristics of the pial arterioles indicate that they are less permeable than the peripheral arterioles but more permeable than the intracerebral arterioles. The pial arterioles *in vivo* demonstrate vasomotor responses to hypercapnia (dilatation), hypoxia (dilatation), hypertension (constriction) and hypotension (dilatation) that are characteristic of the cerebral circulation [Rosenblum & Kontos, 1974; Paulson et al., 1990]. The responsiveness of pial arterioles to physiologic alterations have formed the

basis of the justification of the use of these vessels for the examination of vasomotor responses *in situ*.

The *in situ* techniques for the measurement of cerebrovascular responses have a number of advantages compared to the *in vitro techniques*. The *in vitro* techniques are limited by the size of vessel and the level of the arterial tree that can be examined. The *in situ* techniques facilitate the measurement of the vascular responses of cerebral resistance arterioles with a wider range of diameters (<30 μm) than permitted by the *in vitro* techniques [Wahl et al., 1972; data from present investigations]. The vasodilatation of pial arterioles can be elicited in the absence of preconstriction and the inherent pharmacological interactions that may exist. The pial arterioles examined *in situ* are in their normal physiologic milieu and neurogenic control [Wahl et al., 1972]. The techniques used *in situ* permits the rapid multiple measurement of the diameter of the resistance arteriole (approximately 2 sec) and facilitates the understanding of a time course of the development of the response especially for long acting peptides such as endothelins [Robinson & McCulloch, 1990]. The use of single concentrations of transmitters or peptides facilitates the understanding of the $t_{1/2}$ of the agent *in vivo*. In *in vitro* investigations the potential absence of uptake and degradation mechanisms as a result of the dissection process may prevent an accurate estimation of the half life.

There are essentially two *in situ* techniques that have been developed for the examination of the vasomotor responses of cerebral arterioles. The open skull preparation was developed by Wahl and colleagues in Munich (1970). A craniectomy is performed and the underlying dura is resected to expose the cortical surface [Wahl et al., 1970]. The cerebral microenvironment is protected by a pool of mineral oil and the agents are applied by a subarachnoid microinjection into the perivascular space. Microlitre quantities of the substances are administered locally around the arteriole and the vessel calibre is measured using an image splitting technique [see material and methods]. In the closed skull preparation, a craniectomy is performed and the dura resected and a sealed transparent window placed over the craniectomy site. The technique was developed by Raper and colleagues in Virginia (1971). The cerebral microenvironment underlying the cranial window is superfused with artificial CSF and

the pH and oxygenation of the CSF is controlled. The substances added to the CSF and superfused over the cortical surface and the vascular diameter measured using image splitting techniques. The closed skull technique has been modified to include laser doppler probes to permit the monitoring of cerebral blood velocity in the underlying cortex.

There has been some controversy over the relative merits of the use of open and closed skull preparations. There have been suggestions that there is marked alkalosis in the underlying cortex in the open skull preparation [Navari et al., 1978]. However, the investigations of Kuschinsky and Wahl (1979) have demonstrated that the pH of the underlying cortex is maintained by the mineral oil (the open skull preparation used in the present thesis is as described by Wahl et al., 1970). The action of the mineral oil as a concave lens has been suggested and has been refuted. The responses of cerebral arterioles to hypercapnia and hypotension are similar in the open and closed skull preparations [Raper et al., 1971; Kontos et al., 1978; MacKenzie et al., 1979; Gregory et al., 1980]. The exposure of the cortical brain surface may lead to herniation through the craniectomy in the open skull preparation. The open skull preparation, as described by Wahl and colleagues (1970), has frequently been used to examine feline cortical cerebral arterioles. There is no evidence for herniation of the feline brain but the use of the open skull preparation in smaller species such as the rat may lead to herniation of the brain over a prolonged experimental period. In species such as the rat it may be beneficial to use the closed skull preparation where a positive intracranial pressure can be maintained.

The differences in the vasomotor responses of the cerebral arterioles in the open and closed skull preparations has been attributed to a number of differences in the methodology rather than the intrinsic differences in the responses of the cerebral arterioles to the agents [Navari et al., 1978; Kontos et al., 1980; Kuschinsky & Wahl, 1980]. An important difference in the *in situ* and *in vitro* techniques is the use of anaesthetic agents. The common anaesthetic agents used in *in vivo* experimental studies are halothane and the barbiturates. There have been a number of investigations that have demonstrated that the barbiturates and halothane alter cerebral blood flow

and the resting vasomotor tone (by inducing a vasodilatation) [Fitch et al., 1983; Bonvento et al., 1994]. The observed differences in the actions of vasoactive agents such as potassium can be attributed to the anaesthetic regimen used. The investigations of Fitch et al. (1983) have demonstrated the marked differences in the vasomotor response of cerebral arterioles to potassium in barbiturate and chloralose anaesthetised cats. In the present thesis, the examination of vasomotor responses of cerebral arterioles were carried out under chloralose anaesthesia.

Cerebral blood flow and the calibre of cerebral resistance arterioles is affected by alterations in the local metabolic balance [MacKenzie et al., 1976; Harper & MacKenzie, 1977; Paulson et al., 1990;]. In investigations using superfusion, alterations in cerebral arteriolar calibre have been attributed to the agents. Noradrenaline, 5-Hydroxytryptamine, vasoactive intestinal polypeptide and NMDA have demonstrated increases in cerebral arteriolar calibre [MacKenzie et al., 1976; Harper & MacKenzie, 1977]. These observations have been attributed to the activation of the respective receptor subtypes on the cerebral arterioles. However, the superfusion of the agents does not permit the isolation of the vascular responses that can be attributed to receptors on the arterioles and effects that are the result of the metabolic alterations. The agents administered by superfusion can act on receptors that are present on neurones in the underlying cortex and the alterations in vasomotor responses may be due to the alterations in cerebral metabolic rate.

In the open skull preparation, substances are applied by subarachnoid microinjection into the perivascular space. The microlitre quantities are applied locally in the vicinity of the arteriole. The concentration of the vasoactive agents is maximum at the site of injection and can be attributed to the effects on the cerebral arterioles. The agent is diluted in the perivascular space and thus minimal amount of the agents will act on the cortical surface. The existence of the perivascular space means that the concentrations of drugs described are those in the micropipette and the exact concentration at the receptor is unclear.

The *in situ* techniques have been important in the identification of the vasomotor effects of vasoactive peptides such as CGRP, Vasoactive Intestinal

Polypeptide and endothelins [see section 1.1.3 and 1.3]. The closed and open skull preparations have been used for examining the effect of Vasoactive Intestinal Polypeptide on cerebral arterioles. The investigations demonstrated that vasoactive intestinal polypeptide was approximately 10 fold more sensitive in the open skull preparation that the closed skull preparation in the cat [McCulloch & Edvinsson, 1980]. A comparison of responses of cerebral arterioles in the cat to the superfusion and microapplication of endothelin-1 has not been carried out. Observations in the rat indicate that higher concentrations of endothelin-1 are required to constrict cerebral arterioles using the superfusion method rather than the microapplication method [Salom et al., 1995]. The differences in responses of peptides in the closed and open skull preparation could be explained by the arachnoid membrane limiting access to the cerebrovascular smooth muscle when the peptide molecules are superfused over the cortical surface. In the open skull preparation the peptides are delivered by perivascular microapplication directly to their site of action.

A fundamental difference between the *in situ* and *in vitro* techniques is the size of cerebral vessels that can be examined. The *in vitro* techniques have been used for examining the vasomotor responses of the larger cerebral arteries. However, the pial arterioles are the major contributors to cerebrovascular resistance and the regulation of cerebral blood flow. The use of *in situ* techniques has provided evidence for the dependence of arteriolar response on the size of arteriole examined [Wahl et al., 1972; Harper & MacKenzie , 1977; McCulloch & Edvinsson, 1980]. The smaller pial arterioles (<100 μm) demonstrated greater reactivity to vasodilatation induced by Vasoactive Intestinal Polypeptide, 5-HT and the endothelin ET_B receptor agonist BQ-3020 [Harper & MacKenzie , 1977; McCulloch & Edvinsson, 1980; present thesis]. The larger pial arterioles demonstrated greater reactivity to vasoconstriction elicited by 5-HT and noradrenaline. The size limitations of the *in vitro* techniques would not have facilitated the identification of the altered reactivities of cerebral arterioles.

The *in vitro* techniques allow the isolation of single receptor subtype and the examination of the vasomotor responses of cerebral arterioles following the exposure to the drug. The *in vitro* techniques allow detailed pharmacological analysis to be

carried out and multiple dose response curves to be performed on the same segment of cerebral artery. The pharmacology of receptor antagonists such as the endothelin ET_A receptors can be characterised *in vitro* preparations. The *in situ* techniques do not permit detailed pharmacological analysis on the cerebral arterioles.

Species differences in the responses of cerebral vessels is an important issue in research. The *in situ* techniques permit the examination of the same vessel from a variety of species. In endothelin research, *in situ* investigations have been performed on cerebral vessels from a variety of species (see Table 14 for references). There are no significant differences in the EC_{50} values for endothelin-1 in the major cerebral arteries (see Table 12). The species that can be used in *in vitro* investigations are limited by the size of vessel. However, the majority of in *in situ* investigations have been carried out in the cat and the pial arterioles are commonly examined from the territory of the middle cerebral artery. The most commonly used species in endothelin research are the cat and the rat. The vasomotor responses of cerebral arterioles to endothelin-1 and endothelin receptor agonists have been characterised, however, there are few investigations examining the vasomotor responses of cerebral arterioles form the rat.

Following the identification of the pharmacological characteristics of potential therapeutic agents, the next stage is the evaluation of the pharmacological effects of the therapeutic agents on the cerebrovasculature. The existence of the blood-brain barrier and the ability of a drug to penetrate the blood-brain barrier has important implications for drug development. The *in situ* techniques do not permit the assessment of such properties. The *in situ* techniques, especially the open skull preparations, facilitate the examination of the effects of therapeutic agents when they administered systemically. In the present thesis we have examined the ability of the endothelin receptor antagonists to attenuate the vasoconstrictor actions of exogenous endothelin-1. We have used the *in situ* open skull preparation to examine the vasomotor effects of endothelin receptor antagonist following middle cerebral artery occlusion. The ischaemic conditions cannot be modelled *in situ* preparations, although investigators have exposed arteries to hypoxia. The combination of *in vitro* and *in situ*

techniques permits the characterisation of the endothelin receptors and the impact of endothelin receptor activation on cerebral blood flow under normal and pathological conditions.

4.1.2 Endothelin Receptor Antagonists

The development of endothelin receptor antagonists was important for understanding the role of endothelins in vascular pathophysiology. The first antagonists described were natural by-products of the fermentation of *Streptomyces misakiensis* (BE-18257B) [Ihara et al., 1991]. This compound demonstrated low potency at endothelin receptors in binding and functional bioassays (IC_{50} endothelin ET_A 1.4 μM; endothelin ET_B >100 μM) [Ihara et al., 1991]. Structure activity relationship studies have led to modifications of the peptide structures of the endothelins and to the development of a series of peptide endothelin ET_A, endothelin ET_B and combined endothelin ET_A/ET_B endothelin receptor antagonists [Ishikawa et al., 1994, Ihara et al., 1991; Ihara et al., 1992; Cody et al., 1993; Itoh et al., 1993]. BQ-123 is the most potent of this series and selective for the endothelin ET_A receptor [Ihara et al., 1992]. The IC_{50}'s in binding studies of some of the compounds used in cerebrovascular investigations are described in Table 2.

The distinguishing feature of the cerebral circulation is the blood-brain barrier. The blood-brain barrier is formed of tight junctions between endothelial cells and prevents paracellular transport of large and charged molecules [Edvinsson et al., 1993]. The absence of pinocytotic vesicles prevents transcellular transport of molecules across the blood-brain barrier [Ermisch et al., 1993]. The presence of the blood-brain barrier means that therapeutic agents, such as the endothelin receptor antagonists, that are targeted at the brain must penetrate this barrier to gain access to the cerebrovascular smooth muscle and neurones. There are a number of disadvantages in the use of peptide endothelin receptor antagonists as therapeutic agents for cerebrovascular diseases. The peptide antagonists would undergo rapid hydrolysis by peptidases present in the systemic circulation or in the gastro-intestinal tract if administered intravenously or orally. More importantly, bioactive amounts of peptide endothelin antagonists do not gain access to the adventitial surface of cerebral arterioles when administered systemically [Patel et al., 1995; Clozel & Watanabe, 1993]. The importance of blood-brain barrier permeability for endothelin antagonists

was demonstrated by the failure of intravenous administration of peptide endothelin receptor antagonist (BQ-123) to attenuate cerebral vasospasm or vasoconstrictor action of exogenous endothelin-1 applied to cerebral arterioles [Clozel & Watanabe, 1993].

Recently there has been the development of non-peptide endothelin receptor antagonists (see Table 2). The first non-peptide endothelin receptor antagonist described was Ro46-2005 and its therapeutic potential was demonstrated in experimental subarachnoid haemorrhage [Clozel et al., 1993]. A number of other potent non-peptide endothelin receptor antagonists selective for the endothelin ET_A and combined endothelin ET_A/ET_B receptors have been developed (see Table 2). We have examined the ability of non-peptide endothelin receptor antagonists to gain access to the cerebrovascular smooth muscle and attenuate the vasoconstriction induced by the adventitial application of endothelin-1 and demonstrated the potential of these antagonists as therapeutic agents [present thesis]. However, the non-peptide structure of an endothelin receptor antagonist does not necessarily confer blood-brain barrier permeability (e.g. SB209670) [Ohlstein et al., 1994].

The attenuation of the effects of endogenous endothelins in cerebrovascular disease may also be achieved by endothelin converting enzyme inhibitors. The cDNA's encoding endothelin converting enzyme(s) (ECE) have recently been cloned and will facilitate the development of enzyme inhibitors selective for ECE [Schmidt et al., 1995; Deschepper et al., 1995]. In contrast to the rapid development of endothelin receptor antagonists, only a few ECE inhibitors have been developed e.g. Phosphoramidon, CGS26303, CGS26393 [Kukami et al., 1994; De Lombaert et al., 1995]. The utility of the ECE inhibitors has been examined in preliminary studies in focal cerebral ischaemia [Duverger et al., 1993].

The efficacy of peptide and non-peptide endothelin receptor antagonists in attenuating the responses of exogenous endothelin-1 and in pathophysiologic conditions has been demonstrated. In the cerebrovasculature, the endothelin receptor antagonists have attenuated vasospasm following subarachnoid haemorrhage but the antagonists were administered directly into the cisterna magna thus circumventing the

blood-brain barrier [Clozel & Watanabe, 1993; Cosentino et al., 1993; Nirei et al., 1993; Itoh et al., 1993; Itoh et al., 1994; Hirose et al., 1995]. The intravenous administration of the peptide antagonist BQ-123 had no effect on post subarachnoid haemorrhage induced cerebral hypoperfusion [Clozel & Watanabe, 1993]. In the cerebrovasculature the ability of the endothelin receptor antagonist to penetrate the blood-brain barrier is essential for their therapeutic utility.

The permeability of the blood-brain barrier alters as the arterial tree progresses [Yamashita et al., 1985]. The main inflow vessels, the carotid arteries and the basilar artery, have been demonstrated to possess an endothelial barrier that is permeable to horseradish peroxidase [Yamashita et al., 1985]. In conditions of subarachnoid haemorrhage the integrity of the blood-brain barrier may be compromised as a result of the rupture of the cerebral vessel or damage to endothelium caused by blood borne substances such as thrombin. At the level of the pial resistance arterioles on the cortical surface the evidence suggests that the blood-brain barrier may be in a transitional zone and permeable to small neutral amino acids [Blasberg et al., 1983]. The investigations in the thesis compared the ability of a hexapeptide endothelin receptor antagonist, PD145065 and a non-peptide endothelin receptor antagonist PD155080 to attenuate the vasoconstrictor actions of endothelin-1 following the intravenous administration of the agents.

In our investigations the intravenous administration of the non-peptide endothelin receptor antagonist PD155080 effected a dose dependent attenuation of exogenous ET-1 induced vasoconstriction. In contrast, the intravenous administration of the hexapeptide endothelin receptor antagonist PD145065, did not alter the vasoconstriction elicited by exogenous ET-1. These observations indicated the inability of the hexapeptide endothelin receptor antagonist to gain access to the cerebrovascular smooth muscle to exert its effects. The access to the abluminal surface of the cerebral resistance arterioles critical for the endothelin receptor antagonists to exert their effects. However, access of the non-peptide antagonist to the parenchyma may be more important in pathophysiologic conditions. We have demonstrated that following intravenous administration approximately 10-12% of the plasma

concentration of an analogue, PD156707, can be detected in the CSF aspirated from the cisterna magna [T. R. Patel, unpublished observations].

The intravenous administration of the hexapeptide antagonist PD145065 could have resulted in its inactivation by circulating peptidases or by peptidases present in the vascular endothelium [Rubanyi & Polokoff, 1994]. In our investigations we administered ET-1 as an intravenous bolus injection 120 min. following the intravenous administration of PD145065. The transient reduction in the MABP elicited by ET-1 and the ET-1 induced increase in MABP was attenuated by the dose of PD145065 used in the present investigations. These observations indicated that hexapeptide PD145065 was able to attenuate the haemodynamic responses of exogenous ET-1 in the periphery and the inability of PD145065 to attenuate the ET-1 induced vasoconstriction of pial resistance arterioles was not the result of the inactivation of the hexapeptide antagonist or the inadequacy of the dose of PD145065 used.

The intravenous doses of the non-peptide receptor antagonist PD155080 and the hexapeptide antagonist PD145065 were selected from the *in vivo* and *in vitro* pharmacology of the compounds previously described [Cody et al., 1993; Doherty et al., 1995]. The dose of PD145065 selected in the present investigations (30 µmol/kg) was 3 fold greater than IC_{50} concentration required for the attenuation of the haemodynamic responses of exogenous ET-1 in the rat [Cody et al., 1993]. The intravenous dose of PD155080 used in the present investigations (19.2 µmol/kg) was the IC_{50} dose for the attenuation of the vasoconstrictor response of ET-1 (1 nmol/kg) in the rat [Doherty et al., 1995]. The transient alterations in blood pressure observed during the intravenous injection of choline salt of PD155080 were also observed during the intravenous injection of choline chloride. The mechanism underlying these transient alterations in blood pressure are unclear, but, it has been suggested that choline may act by increasing central cholinergic neurotransmission and activate central mechanisms involved in the regulation of blood pressure [Ulus et al., 1995].

The pharmacology of Bosentan (Ro 47-0203) has recently been characterised in detail [Clozel et al., 1994] and it shares many similarities with the structurally

related agent Ro 46-2005 [Clozel et al., 1993]. Bosentan is a competitive antagonist at endothelin ET_A and endothelin ET_B receptors. In isolated tissues, Clozel and colleagues (1994) have shown that Bosentan inhibits the action of endothelin at endothelin ET_A receptors (rat aorta), ET_{B1} receptors (receptor mediating vasodilatation in the rabbit superior mesenteric arteries) and the ET_{B2} receptors (receptor mediating vasoconstriction in rat tracheal rings) with pA_2 values of 7.28, 6.72 and 5.94 respectively. In that study the *in vitro* demonstration of the competitive antagonism of the ET-1 induced vasoconstriction in the rat aorta by Bosentan at doses of 0.3 - 3 μM, is in accordance with the antagonists dose range utilised perivascularly in the present study. With systemic dosing, similar to that employed in the current study, Bosentan *per se* did not alter blood pressure in the pithed rat [Clozel et al., 1994]. However these authors did demonstrate that intravenously administered Bosentan (3-30 mg/kg) dose-dependently attenuated the initial vasodepressor and subsequent vasopressor effects of intravenous ET-1 (0.03 -1 nmol/kg).

The present studies investigated 3 distinct issues. First, co-application of Bosentan, PD155080 and PD145065 onto the adventitial surface has been shown to attenuate the vasoconstriction induced by exogenous ET-1 and that the concentrations required in the present study are in accord with those in peripheral vascular tissues *in vitro* [Cody et al., 1992; Clozel et al., 1994; Doherty et al., 1995]. Second, the microapplication of Bosentan, PD155080 and PD145065 *per se* (at concentrations which prevent ET-1 induced vasoconstriction) had no effect on pial arteriolar calibre. Such data are consistent with the view that, at least at the level of the adventitial surface, there is minimal tone due to endogenous endothelins under normoxic, normocapnic, normotensive, non-pathologic conditions. The available evidence from the present study suggests that exogenous endothelins can act at the level of the adventitial surface in cerebral vessels. Third, the present data provide evidence that sufficient Bosentan and PD155080 gains access after intravenous administration to the vicinity of pial arterioles to attenuate the constrictions induced by topical application of ET-1 (10 nM). The ability to manipulate cerebrovascular endothelin receptors with

systemic drug administration will facilitate the elucidation of the role of endothelins in cerebrovascular pathology.

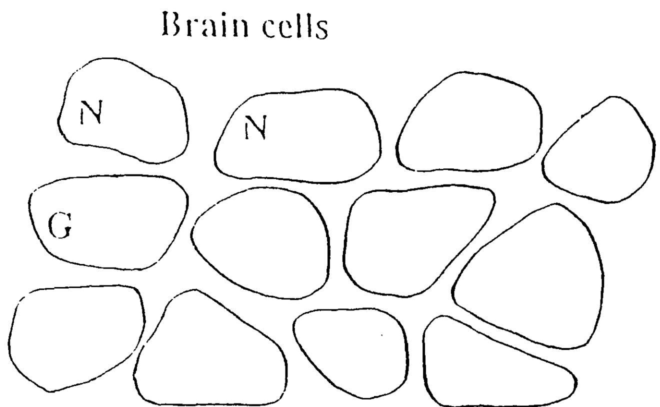
The endothelin receptor subtype (s) which mediate the constriction of cerebral blood vessels to endothelin has been the subject of limited investigation. The available evidence suggests that endothelin constricts the major cerebral arteries of the guinea pig and goat via an endothelin ET_A receptor [Adner et al., 1993; Salom et al., 1993]. Our investigations point to the endothelin ET_A receptor subtype mediating ET-1-induced cerebral vasoconstriction in feline pial arterioles *in situ*. Irrespective of the roles of different endothelin receptor subtypes, the present studies suggested that Bosentan and PD155080 (applied topically or systemically) would be valuable tools in the elucidation of the functional significance of endothelins in the cerebral circulation *in vivo* [Figure 44].

Concluding Remarks

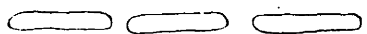
The therapeutic utility of the endothelin receptor antagonist depends on their blood-brain barrier permeability. The demonstration in these investigations that the non-peptide endothelin receptor antagonist attenuate the vasoconstriction elicited by exogenous endothelin-1 provides evidence that the non-peptide antagonists penetrate the blood brain barrier [Figure 44]. However, we must be cautious when interpreting these investigations because there is evidence that the cortical surface arterioles are more permeable to small neutral amino acids than the intraparenchymal arterioles (but less permeable than peripheral blood vessels. The critical observation in such investigations would be the detection of drug concentrations in the CSF following their systemic administration. Recent investigations have indicated that in the cat 10-12% of the systemic dose of PD156707 was detected in the CSF while in the rat approximately 1% of the systemic dose of Bosentan was detectable in the CSF [Patel & McCulloch, unpublished observations; M. A. McAuley, unpublished observations]. These observations have strengthened arguments for using these compounds for examining the role of the endogenous endothelins in pathophysiology.

Figure 44 Schematic representation of the cerebral circulation. The gap junctions present at the level of the major cerebral arteries may permit access of peptide endothelin receptor antagonists to the cerebrovasculature. However, the peptide endothelin receptor antagonists fail to gain access to the cerebrovascular smooth muscle of pial and intracerebral arterioles.

Endothelin Receptor Antagonists
Blood Brain Barrier Permeability



Cerebrovascular smooth muscle



Endothelium

Major Arteries	Pial Arterioles	Intraparenchymal arterioles and capillaries
Partial Blood Brain Barrier	Intact Blood Brain barrier	Intact Blood Brain barrier

Barrier Permeability

Peptide Antagonists?	Non Peptide Antagonists
Non peptide Antagonists	(bosentan, PD155080, PD156707)

4.2 Pathophysiological role of endothelin and endothelin receptor antagonists

The potent constrictor actions of endothelins have implicated them in a variety of vascular and non vascular pathologies [Rubanyi & Polokoff, 1994]. There is evidence for a role of the endothelins in cardiac ischaemia, renal ischaemia and hypertension [Rubanyi & Polokoff, 1994; Gandhi et al., 1994; Gellai et al., 1994; Brunner et al., 1992]. However, the discussion of pathophysiological roles of the endothelins in the periphery is outside the scope of the present article [Rubanyi & Polokoff, 1994; Gandhi et al., 1994].

The application of exogenous endothelin-1 to neuronal cell cultures failed to demonstrate a neurotoxic effect [Lustig et al., 1992; Nikolov et al., 1993]. The administration of exogenous endothelin-1 into the cerebral ventricles following focal cerebral ischaemia appeared to exacerbate the ischaemic damage in the mouse [Nikolov et al., 1993]. Topical application of exogenous endothelin-1 on to the middle cerebral artery in the rat is associated with a marked reduction in cerebral blood flow and ischaemic neuronal damage within the cortex and striatum [Fuxe et al., 1992, Robinson et al., 1990; Macrae et al., 1993; Sharkey et al., 1993]. These effects demonstrate the ability of endothelin-1 to overwhelm the protective homeostatic mechanisms that exist in the brain to maintain blood flow and induce neuronal damage.

4.2.1 Experimental Cerebrovascular Diseases

Global Ischaemia

Transient global cerebral ischaemia is characterised by a marked reduction in cerebral blood flow followed by transient hyperaemia and delayed hypoperfusion [Hossmann et al., 1973; Miller et al., 1980; Pulsinelli et al., 1982; Kagstrom et al., 1983]. The onset of post ischaemic hypoperfusion is rapid after the transient hyperaemic response, and the mechanisms mediating post ischaemic hypoperfusion, despite much speculation, remain unclear [Miller et al., 1980; Frerichs et al., 1992]. An increase in vascular tone, obstruction of the cerebral microvasculature, as a result of blood aggregation or constriction of cerebral vessels as result of tissue oedema or glial swelling, are but some of the mechanisms proposed [Grogaard et al., 1989; Moskowitz et al., 1990; Wahl & Schilling, 1993; del Zoppo, 1994; Dirnagl et al., 1994].

The phenomenon of post-ischaemic hypoperfusion has been investigated following transient focal and global ischaemia in the rat and the cat [Hossmann et al., 1973; Miller et al., 1980; Pulsinelli et al., 1982; Kagstrom et al., 1983]. There are a number of factors that can be involved in the maintenance of hypoperfusion such as increased vascular tone or microvascular plugging [Wade et al., 1975; Grogaard et al., 1989; Moskowitz et al., 1990; Wahl & Schilling, 1993; Dirnagl et al., 1994]. This investigation failed to demonstrate any increases in the endothelin mediated vascular tone during post-ischaemic hypoperfusion. The vascular mechanisms involved in post-ischaemic hypoperfusion have been investigated using the calcium channel antagonist nimodipine [Kazda et al., 1982; Steen et al., 1984]. The ability of nimodipine to alleviate hypoperfusion indicates an increase in vascular tone of cerebral vessels during the post-ischaemic period [Kazda et al., 1982]. This attenuation of post-ischaemic hypoperfusion has been associated with increases in neuronal survival and neurologic outcome in experimental models of global ischaemia [Steen et al., 1984].

The endothelins have been implicated in cerebrovascular pathology [Rubanyi & Polokoff, 1994]. Increases in tissue endothelin immunoreactivity have been measured following global and focal ischaemia [Duverger et al., 1992; Viossat et al.,

1993; Barone et al., 1994]. Increases in plasma and CSF endothelin levels have been reported in subarachnoid haemorrhage, stroke and global ischaemia at shorter time points [Suzuki et al., 1992; Ziv et al., 1992; Barone et al., 1994; Giuffrida et al., 1990; Giuffrida et al., 1992; Willette et al., 1992].

The endothelin ET_A receptor antagonists BQ-123 and SB209670 have demonstrated a decrease in neuronal damage in hippocampal CA1 neurones in a gerbil model of global ischaemia [Feuerstein et al., 1994; Ohlstein et al., 1994] (see Table 15). The mechanism underlying this process is unclear but an increase in cerebral blood flow and direct neuronal effects of the endothelin receptor antagonists are possibilities. This investigation did not attempt to investigate the effect of Bosentan on neuronal damage following transient global ischaemia in the acute phase. Recently Yamashita and colleagues (1994) have demonstrated an increase in endothelin ET_B receptors in CA1 pyramidal neurones following transient forebrain ischaemia. The increase in endothelin receptors is associated with microglia and the role of endothelins in the progression of neuronal cell death following forebrain ischaemia is unclear. The investigations in the present thesis were designed to examine if delayed hypoperfusion associated with transient forebrain ischaemia could be modified by the combined endothelin ET_A/ET_B receptor antagonist Bosentan.

The vasoconstrictor actions of endothelins are mediated primarily via endothelin ET_A receptors although constrictor endothelin ET_B receptors have been reported [Seo et al., 1994; Rubanyi and Polokoff, 1994]. Recent data has indicated the presence of endothelin ET_B receptors in the cerebral resistance arterioles that mediate vasodilatation [Kobari et al., 1994; present thesis]. Bosentan is a combined endothelin ET_A/ET_B receptor antagonist and the absence of any increases in cerebral blood flow during post-ischaemic hypoperfusion could be the result of the inhibition of the dilator endothelin ET_B receptor and the constrictor endothelin ET_A receptor [Clozel et al., 1994]. The increases in endothelin mediated tone in cerebral resistance arterioles following focal cerebral ischaemia in the cat indicates a regulatory role for the endothelin peptides during the post-ischaemic phase [present thesis]. However, Bosentan failed to increase cerebral blood flow or reduce the volume of damaged

Table 15

Effect of endothelin receptor antagonists in animal models of global and focal cerebral ischaemia

Model/species	Compound (dosage)	Administration schedule (route)	Outcome	Reference
Global Cerebral Ischaemia				
Gerbil	BQ-123 (10μg)	5 min pre & 60 min post induction of global; ischaemia; neuronal counts at 7 days post-ischaemia (i.c.v.)	21% Decrease CA1 neurones (61% decrease in CA1 neurones in vehicle treated animals)	Feuerstein et al., 1994
	SB209670 (50μg)	Administered 5 min pre & 60 min post ischaemia; neuronal counts 7 days post-ischaemia (i.c.v.)	6% Decrease CA1 neurones (55% decrease in CA1 neurones in vehicle treated animals)	Ohlstein et al., 1994
Rat	Bosentan (17 μmol/kg)	15 min prior to induction of ischaemia; CBF measured pre & post-occlusion using autoradiography and hydrogen clearance) (i.v.)	No effect on post-ischaemic hypoperfusion	Present Thesis
Focal Cerebral Ischaemia				
Rat	Bosentan (25.5 μmol/kg)	15 min prior to MCAO; infarct measured 4 hours post MCAO) (i.v.)	marginal decrease in volume of infarct (17%)	McAuley et al., 1994
	BQ123 (23 μmol/kg/hr)	30 min pre-ischaemia; infarct measured 24 hours post MCAO (i.v.)	no effect	Checkley et al., 1995
	Phosphoramidon (3 mg/kg)[a]	15 min & 5 hours following MCAO; infarct measured 48 hours post occlusion (i.v.)	30% decrease in volume of hemispheric infarct	Duverger et al., 1993
Rat (SHR)	SB217242 (15 mg/kg)	7 days prior to MCAO; Infarct measured 24 hours post-MCAO) (p.o.)	decrease in volume of infarct (30%)	Barone et al., 1995
	BQ123 (23 μmol/kg/hr)	30 min pre-ischaemia; infarct measured 24 hours post MCAO (i.v.)	decrease in volume of infarct (25%)	Patel et al., 1995

Table 15

Cat	Bosentan (30μM)	Around individual arterioles 30-210 min following MCAO (Topical)	29% increase in post-occlusion arteriolar diameter	Present Thesis
	PD155080 (30μM)	Around individual arterioles 30-210 min following MCAO (Topical)	21% increase in post-occlusion arteriolar diameter	Present Thesis
	PD156707 (3 μmol/kg bolus + 5 μmol/kg/hr infusion)	Administered 30 min following MCAO; infarct measured 6 hours post MCAO (i.v.)	decrease in volume of infarct (45%) Increase in penumbral CBF (to pre-occlusion baseline levels)	Present Thesis

[a] Endothelin-converting enzyme inhibitor
Abbreviations: CBF (cerebral blood flow); MCAO (middle cerebral artery occlusion); i.v. (intravenous); i.c. (intracisternal); i.c.v. (intracerebroventricular); p.o. (oral)

tissue in focal ischaemia in the rat [McAuley et al., 1994]. Similarly, the inability of Bosentan to increase cerebral blood flow following transient global ischaemia indicates that the contribution of endothelins in mediating post-ischaemic vascular tone may be less pronounced in the rat. The reasons for such differences are not immediately clear but differences in distribution or density of endothelin receptor subtypes in the rat and cat are possibilities [Kobari et al., 1994; Feger et al., 1994]. There is no clear evidence to suggest that differences in endothelin receptors may exist between these two species. Other possible explanations include the differences in the cerebrovascular anatomy of the rat and cat [Coyle & Jokelainen, 1982; Mchedlishvili & Kuridze, 1984]. The lower density of connections between collateral vessels in the cerebral cortex of the rat is one possible explanation for the failure of Bosentan to increase cerebral blood flow following transient global ischaemia [Coyle & Jokelainen, 1982; Mchedlishvili & Kuridze, 1984].

The source of endothelins following cerebral ischaemia is unclear [see section 4.2.2]. Endothelin receptor binding sites and mRNA have been described on neuronal and glial cells [MacCumber et al., 1990; Ehrenreich et al., 1991]. There are a number of possible triggers for the synthesis of endothelin peptides, e.g. tissue hypoxia and thrombin [Kurihara et al., 1989]. Tissue hypoxia during the period of global ischaemia and the pooling of blood in the microvasculature could be possible triggers for the release or synthesis of endothelins. The role of vascular elements such as leucocytes and neutrophils in microvascular obstruction has been investigated [Wahl & Schilling, 1993; Dirnagl et al., 1994]. The haemorrhage and the subsequent re-infusion of blood could cause trauma to the cellular contents and increase the ability of platelets to aggregate and increase leucocyte adhesion to vascular endothelium [Grogaard et al., 1989; Dirnagl et al., 1994]. The increased trauma to blood cells such as the platelets can result in the release of vasoconstrictor factors or an increase in endothelin-1 mRNA and thus increase vascular tone [Kurihara et al., 1989]. The haemorrhagic hypotension employed to reduce cerebral blood flow could by itself result in the increase in systemic endothelin release [Zimmermann et al., 1994]. An increase in the shear stress on endothelial cells has been shown to stimulate

increases in endothelin release [Malek & Izumo, 1992]. The transient hyperaemic response immediately following the restoration of cerebral blood flow may be one of the triggers for increased endothelin release or production. The transition from transient hyperaemia to delayed hypoperfusion is a rapid event (approximately over 5 minutes). The mechanism mediating the rapid transition is unclear but Moskowitz and colleagues (1990) have suggested that there is a loss of dilator vascular tone. Pial cerebral arterioles have perivascular nerves containing dilator peptides such as CGRP and sudden loss of tone leads to the constriction of vessels and reduction in cerebral blood flow. We have demonstrated an increase in endothelin mediated vascular tone in vessels following middle cerebral artery occlusion in the cat [present thesis]. However, the inability of Bosentan to alleviate the hypoperfusion following transient global ischaemia indicates that the endothelins may not play a major role in the maintenance of hypoperfusion.

In a pharmacological investigation which fails to reveal a significant effect, the issue of adequacy of experimental design is crucial to the interpretation of the results. The dose of Bosentan (17 μmol/kg) utilised in the present investigation has been demonstrated to significantly reduce the pressor effect of exogenous ET-1 in the rat by approximately 40 mmHg (ED_{50} dose) [Clozel et al., 1994]. Our own investigations in feline pial arterioles have demonstrated that this dose of Bosentan completely inhibits the vasoconstrictor response following the perivascular application of ET-1 (10 nM) [Patel et al., 1994]. However, differences in the reactivity of feline pial arterioles and rat intracerebral microvessels to endothelins or endothelin receptor antagonists cannot be excluded absolutely [Robinson & McCulloch, 1990; Feger et al., 1994; Sagher et al., 1994]. The plasma half-life of Bosentan is approximately 3 hrs [Clozel et al., 1994], thus, the absence of an effect may not be due to rapid clearance of the drug from the circulation. The issue of the adequacy of experimental design was addressed by post hoc statistical power analysis. In the Iodoantipyrine autoradiography investigations, the present studies would permit the significant detection of a 40% increase in CBF following treatment with Bosentan. In the hydrogen clearance studies a 30% increase in CBF would be detected significantly with the current experimental

design. The studies were carried out using two different techniques for measuring cerebral blood flow, one with excellent spatial resolution ([^{14}C]-Iodoantipyrine autoradiography) and the other with good temporal resolution (hydrogen clearance), and employing two different models of ischaemia, one severe that is associated with marked hypoperfusion (carotid artery occlusion with hypotension) and the other with moderate severity with minimal hypoperfusion (common carotid artery occlusion). The failure of Bosentan to alter acutely the post-ischaemic hypoperfusion does not rule out the possibility of the endothelins playing a role in global cerebral ischaemia.

Subdural Haematoma

Head injury is often associated with ischaemic neuronal damage. the pathophysiologic processes that ensue following the head injury are varied but despite the use of drugs and surgical intervention the prognosis for these patients is poor [Jamieson & Yelland, 1972; Seelig et al., 1981; Stone et al., 1983; Bullock & Fujisawa, 1992]. Neurosurgical intervention involving the removal of a subdural haematoma is often associated with a high risk of mortality (~50%) and neurological disabilities (~20-30%) in those patients that survive [Jamieson & Yelland, 1972; Seelig et al., 1981; Stone et al., 1983; Bullock & Fujisawa, 1992].

Ischaemic neuronal damage following head injury is often the result of cerebral contusions, diffuse axonal injury or hypoxic damage [Graham et al., 1989; Meldrum et al., 1990; Bullock & Fujisawa, 1992]. Reductions in cerebral blood flow have been observed in patients with acute subdural haematomas [Wyper et al., 1991]. The experimental model of subdural haematoma in the rat used in the present thesis displays marked ischaemic neuronal damage (~60 mm^3) confined to the cerebral cortex immediately underlying the haematoma [Miller et al., 1990; Chen et al., 1991b]. The measurement of CBF in the underlying cortex demonstrated a reduction in the blood flow to below 20 ml/100g/min. in ~130 mm^3 of the ipsilateral cerebral hemisphere [Kuroda & Bullock, 1992a; Kuroda & Bullock, 1992b].

Glutamate is the major excitatory amino acid in the brain and elevated levels of glutamate are associated with neuronal damage [Choi, 1990; Benveniste, 1991]. Elevations in extracellular glutamate concentrations have been measured following focal and global cerebral ischaemia [Benveniste, 1991]. The subdural haematoma in the rat is associated with an increase in extracellular glutamate with the peak of the glutamate concentration measured at 45 min. following the haematoma [Bullock et al., 1991]. The elevations in extracellular glutamate concentrations were observed in the cerebral cortex immediately underlying the haematoma and a 3 fold increase in extracellular glutamate was measured in the underlying hippocampus [Bullock et al., 1991]. The elevations in glutamate concentrations were associated with elevations in cerebral glucose utilisation in the molecular layer of the hippocampus and limbic system [Kuroda & Bullock, 1992a; Inglis et al., 1992]. In contrast the cerebral cortex displayed a marked reduction in glucose utilisation [Inglis et al., 1992]. The hippocampus and cerebral cortex subsequently demonstrated ischaemic neuronal damage [Chen et al., 1991b]. The reductions in glucose use in the cerebral cortex were reversed by the intravenous administration of the excitatory amino acid antagonist D-CPP-ene and the hypermetabolism hippocampus was attenuated by D-CPP-ene [Inglis et al., 1992].

The primary insult leading to ischaemic neuronal damage following subdural haematoma is the reduction in CBF in the cerebral cortical areas underlying the haematoma [Miller et al., 1990; Kuroda & Bullock, 1992b]. Autoradiographic studies have demonstrated that the cerebral cortical regions below the haematoma are perfused by CBF at levels that will result in ischaemic damage (~20 ml/100g/min.; see section 1.1.3) [Kuroda & Bullock, 1992b]. The induction of the subdural haematoma is associated with an increase in the intracranial pressure (ICP) but the ICP returns to baseline level 1 hr following the induction of the haematoma [Kuroda et al., 1994]. However, the reductions in CBF are maintained for the duration of the experimental period (typically 2-3 hr) [Kuroda & Bullock, 1992b]. The subdural haematoma is also associated with a marked increase in brain swelling [Kuroda & Bullock, 1992b]. The absence of any alteration in the CBF in the ischaemic territory over the course of the

experimental period suggests that the cerebral oedema is cytotoxic rather than vasogenic in origin [Kuroda & Bullock, 1992b].

The reductions in CBF associated with experimental subdural haematomas are only observed in the presence of subdural blood [Kuroda & Bullock, 1992b]. The use of inert materials , e.g. silicone, to occupy the subdural space have been associated with minimal alterations in the levels of CBF and only a small volume of neuronal damage compared to the animals treated with blood [Miller et al., 1990; Kuroda & Bullock, 1992b]. These observations suggests that the decreases in CBF and associated neuronal damage is combination of the space occupying lesion and vasoactive/cytotoxic mediators released form the blood clot.

The therapeutic interventions in patients with head injury have been confined to the use of excitatory amino acid antagonist or agents to control the elevations in ICP following the haematoma [Kuroda et al., 1994]. In the rat model of subdural haematoma, NMDA receptor antagonists, glycine site receptor antagonists and flow enhancing strategies have been examined with varying degrees of success [Chen et al., 1991b; Kuroda et al., 1994; Tsuchida & Bullock, 1995]. In the rat, pre-treatment with the competitive NMDA receptor antagonist D-CPP-ene reduced the volume of ischaemic neuronal damage associated with the subdural haematoma [Kuroda & Bullock, 1992b]. Similar volume of neuroprotection were afforded by the non-competitive NMDA antagonist MK-801 and NMDA glycine-site antagonist, ACEA-1021 [Kuroda et al., 1994; Tsuchida & Bullock, 1995].

However, the primary trigger for the ischaemic cascade is the reduction in CBF and we examined the efficacy of the combined endothelin ET_A/ET_B receptor antagonist Bosentan. The efficacy of endothelin receptor antagonists in reducing neuronal damage following global and focal cerebral ischaemia has indicated a role for the endogenous endothelins in the progression of neuronal damage [Barone et al., 1995; Feuerstein et al., 1994; Ohlstein et al., 1994; Patel et al., in press]. Vasoactive substances present in the blood, e.g. oxyhaemoglobin, thrombin and platelet derived growth factor, stimulate the synthesis or release of endothelin-1 (see section 4.2.2). Preliminary evidence has also demonstrated an increase in endothelin converting

enzyme mRNA following experimental head injury [Tayag et al., 1995]. The investigations in the thesis were designed to examine the effect of Bosentan on cerebral blood flow following subdural haematoma. Bosentan failed to alter CBF in the cerebral hemisphere ipsilateral to the haematoma. There was no significant difference in the degree of brain swelling in the Bosentan and vehicle treated animals. The failure of Bosentan to have any significant effect on cerebral blood flow could be the result of several possibilities. The severity of the ischaemia following the haematoma resulted in a relatively large volume of tissue perfused by CBF <20 ml/100g/min. (25% of total ipsilateral hemispheric volume). The volume of tissue available for salvage by Bosentan , i.e. tissue which is perfused by CBF for moderate ischaemia (20-50 ml/100g/min.), is markedly less (15% of total ipsilateral hemispheric volume). These volumes of tissues are in contrast to the volume of penumbral tissue available following focal cerebral ischaemia in the rat (CBF <20 ml/100g/min.: 18% of ipsilateral hemispheric volume; CBF 20-50 ml/100g/min.: 22% of ipsilateral hemispheric volume) [Mackay et al., 1993b]. The smaller volume of tissue available for salvage is further compromised by the extensive brain swelling in the ipsilateral hemisphere that is observed at an early time point (2.5 hr). The increased cerebral oedema would be expected to elevate ICP and result in an decrease in the cerebral perfusion pressure [Seelig et al., 1981; Kuroda et al ., 1994]. Tissue oedema in the cerebral cortex would also constrict the intraparenchymal arterioles and the ischaemic lesion may be exacerbated as result of a reduction in the amount of blood flowing through the collateral channels. The ability of endothelin receptor antagonist to reduce ischaemic neuronal damage in focal cerebral ischaemia has been attribute to the ability of these agents to increase cerebral blood flow. The use of flow enhancing strategies like the endothelin receptor antagonist may serve to exacerbate the cerebral oedema associated with subdural haematoma.

The endothelin receptor antagonist used in the present experiment is a combined antagonist for both endothelin ET_A and endothelin ET_B receptors. The receptor distribution of the endothelin receptors in the cerebrovasculature of the rat has indicated the presence of constrictor endothelin ET_A receptors on the vascular smooth

muscle and vasodilator endothelin ET_B receptors on the vascular endothelium [Rubanyi & Polokoff, 1994]. The pharmacology of Bosentan indicates that any beneficial effects resulting from increases in cerebral blood flow by endothelin ET_B receptor activation will be antagonised by Bosentan [Clozel et al., 1994]. There have been reports concerning the accessibility of Bosentan to the adventitial surface of cerebral resistance arterioles following its systemic administration in the rat. We have evaluated the efficacy of systemic administration of Bosentan to antagonist the contractile effects of exogenous ET-1 on cerebral cortical arterioles in the cat [present thesis]. However, there are morphologic differences in the structure of the blood-brain barrier at the level of the surface arterioles and the intraparenchymal arterioles that may account for the ability of bioactive amounts of Bosentan to gain access to the cerebrovasculature (see section 4.1.1). Preliminary investigations examining the blood-brain barrier permeability of Bosentan in the rat indicates that approximately 1% of a intravenous bolus dose is detectable in the cisternal CSF [M. A. McAuley, unpublished observations].

Two important issues that arise from a negative drug study are the adequacy of the drug dosing regimen and the optimisation of the study design. The dose of Bosentan used in the subdural haematoma studies was the ED_{50} dose for the inhibition of the vasoconstrictor actions of intravenous ET-1 (1 nmol/kg) [Clozel et al., 1994]. We have previously demonstrated that bioactive amounts of the dose of Bosentan used in the studies gains access to the cerebrovasculature of the cat. The small sample size in the study and the timing of the measurement of the blood flow measurements would have contributed to the failure to detect an increase in the cerebral blood flow by Bosentan.

Subarachnoid Haemorrhage

The significance of the endothelins in subarachnoid haemorrhage is in its purported role as a mediator of delayed cerebral vasospasm (which normally occurs 3-7 days following the subarachnoid haemorrhage) [Suzuki et al., 1992; Kraus et al., 1991; Fujimori et al., 1990; Yamaura et al., 1992]. The measurement of elevated

levels of endothelin-1 in the plasma and CSF and increases in endothelin immunoreactivity in cerebral vessels following experimental and clinical subarachnoid haemorrhage has implicated endothelin-1 in the development of vasospasm [Suzuki et al., 1992; Kraus et al., 1991; Fujimori et al., 1990; Yamaura et al., 1992; Roux et al., 1995]. The pathological significance of elevated plasma endothelin-1 levels in subarachnoid haemorrhage is unclear since the available evidence indicates that the actions of the endothelins are exerted locally at the site of synthesis or release [Yoshimoto et al., 1990; Wagner et al., 1992] (see Table 16). There is no clear correlation of increases in plasma and CSF endothelin immunoreactivity and the severity of vasospasm.

Increased sensitivity of rat cerebral arteries *in vitro* to endothelin-1 following subarachnoid haemorrhage has been reported [Alafaci et al., 1989]. The mechanism underlying this effect is unclear but is indicative of the role the endothelin peptides may play in the development of vasospasm. Experimental data demonstrating the efficacy of endothelin receptor antagonists in subarachnoid haemorrhage have been described in a variety of species (see Table 16). The peptide endothelin receptor antagonists administered into the cerebral ventricles (BQ-123, BQ-485, FR139317) have demonstrated the efficacy of these compounds in attenuating vasospasm following subarachnoid haemorrhage [Itoh et al., 1993; Clozel et al., 1993; Nirei et al., 1993; Itoh et al., 1994]. The non-peptide endothelin receptor antagonists Ro46-2005 and Bosentan administered orally or intravenously attenuate vasospasm following with subarachnoid haemorrhage [Clozel et al., 1993; Roux et al., 1995; Zimmermann et al., 1995; Shigeno et al., 1995]. In the experimental studies, endothelin ET_A receptor selective and combined endothelin ET_A/ET_B receptor antagonists attenuate vasospasm and there are no demonstrable differences in the efficacy of these sub-groups of receptor antagonists. In the rabbit double haemorrhage model the combined endothelin ET_A/ET_B antagonist PD145065 attenuates vasospasm to a greater degree than the endothelin ET_A antagonist BQ-610 indicating that vasoconstriction in the rabbit basilar artery is mediated by endothelin ET_A and endothelin ET_B receptors. The administration of L-arginine (substrate for the production of nitric oxide) had a

Table 16

Table 16

Effect of endothelin receptor antagonists in animal models of subarachnoid haemorrhage

Model/species	Compound (dosage)	Administration schedule (route)	Outcome	Reference
Subarachnoid Haemorrhage				
Rat (Single Haemorrhage)	BQ123 (10μM)	10 min prior to SAH; CBF measured using microspheres (i.c.)	CBF restored to baseline levels at 60, 120 min	Clozel & Watanabe, 1993
	BQ123 (4.5 μmol/kg)	10 min prior to SAH; CBF measured using microspheres (i.v.)	No alteration in CBF following SAH	Clozel & Watanabe, 1993
	Ro46-2005 (3 mg/kg)	10 min prior to SAH; CBF measured using microspheres (i.v.)	10% decrease in CBF at 60 and 120 min (30% decrease in CBF in vehicle treated animals)	Clozel et al., 1993
Rabbit (Double Haemorrhage)	PD145065 (1μM)	Superfusion on basilar artery ; 4 days after 2nd haemorrhage; vessel diameter measured *in situ*	87% increase in diameter of vasospastic artery	Zuccarello et al., 1993
	BQ610 (1μM)	Superfusion on basilar artery ; 4 days after 2nd haemorrhage; vessel diameter measured *in situ*	45% increase in diameter of vasospastic artery	Zuccarello et al., 1993
	BQ123 (40 nM)	Superfusion on basilar artery ; 2 days after 2nd haemorrhage vessel; diameter measured *in situ*	71% increase in diameter of vasospastic artery	Foley et al., 1994
Rabbit (Single haemorrhage)	Bosentan (51 μmol/kg)	2 days following induction of SAH (i.v.)	36% increase in diameter of basilar artery at day 7	Roux et al., 1995
Dog (double haemorrhage)	FR139317 (0.1 mg)	0, 2, 4 days after induction of SAH (i.c.v.)	14% increase in basilar artery diameter	Nirei et al., 1993
	BQ123 (5 μM/ day)	2 days prior to induction of SAH; vessel diameter measured using angiography at day 7 (i.c.)	Restoration of basilar artery diameter to baseline levels at day 7)	Itoh et al., 1994

Table 16

BQ485 (120 mg/day)	2 days prior to induction of SAH; vessel diameter measured using angiography (i.c.v.)	25% reduction in diameter of basilar artery at 7 days (41% reduction in vehicle treated animals)	Itoh et al., 1993
BQ123 (100μM/day)	30 min prior to induction of SAH; vessel diameter measured using angiography (i.c.)	No significant effect on basilar artery diameter on day 7	Cosentino et al., 1993
BQ123 (1 μmol/kg)	Administered on day 4 & 5 following induction of SAH; vessel diameter measured using angiography (i.c.)	basilar artery diameter 22% of pre SAH baseline at day 5 (37% of pre SAH baseline in vehicle treated animals)	Hirose et al., 1995
Phosphoramidon (200 μM/day) [a]	30 min prior to induction of SAH; vessel diameter measured using angiography (i.c.)	No significant effect on basilar artery diameter on day 7	Hirose et al., 1995
Bosentan (51 μmol/kg/day)	1 day prior to induction of SAH (p.o.)	13% reduction in diameter of basilar artery at day 8 (31% reduction in vehicle treated animals)	Zimmermann et al., 1995
Bosentan (51 μmol/kg)	Day 7 prior to measurement of basilar artery diameter (i.v.)	10% increase in diameter of basilar artery at day 8	Roux et al., 1995
Bosentan (17 μmol/kg, bid)	Immediately following induction of SAH; vessel diameter measured using angiography at day 7 (i.v)	21% decrease in basilar artery diameter at day 7 (49% decrease in diameter in vehicle treated animals)	Shigeno et al., 1995
SB209670 (360 μg/day)	Administered 1 day prior to induction of SAH; vessel diameter measured using angiography (i.c.)	basilar artery diameter 68% of pre SAH baseline at day 7 (27% of pre SAH baseline in vehicle treated animals	Willette et al., 1994

[a]Endothelin-converting enzyme inhibitor

Abbreviations: CBF (cerebral blood flow); MCAO (middle cerebral artery occlusion); SAH (subarachnoid haemorrhage); i.v. (intravenous); i.c. (intracisternal); i.c.v. (intracerebroventricular); p.o. (oral); bid (twice daily)

transient effect on the diameter of the vasospastic basilar artery compared to the sustained increases in diameter effected by the endothelin receptor antagonists [Hirose et al., 1995]. These observations indicate the inactivation of vasodilator nitric oxide/EDRF, e.g. by haemoglobin present in the subarachnoid clot, while the vasoconstrictor actions of the endothelins are unaffected. The majority of investigations have demonstrated the efficacy of endothelin receptor antagonists in experimental subarachnoid haemorrhage, although, the observations of Cosentino et al. (1993), in a canine model of subarachnoid haemorrhage, where BQ-123 and Phosphoramidon failed to alter basilar artery diameter, are in marked contrast to the results obtained in other investigations using a similar model [Itoh et al., 1994; Cosentino et al., 1993; Hirose et al., 1995].

Focal Ischaemia

A persuasive case is developing for the involvement of endothelins in the pathophysiology of focal cerebral ischaemia and for the therapeutic potential of endothelin antagonists [Ziv et al., 1992; Rubanyi & Polokoff, 1994]. The ability of exogenous ET-1 to overwhelm homeostatic mechanisms that maintain cerebral blood flow, even in the normal CNS, has attracted interest in its role in regulating post-ischaemic cerebral blood flow [Robinson et al., 1990; Fuxe et al., 1992; Macrae et al., 1993; Sharkey et al., 1993]. Endothelin receptor antagonists have no significant effect on cerebral arterial or pial arteriolar calibre under non-pathologic conditions *in vivo* nor do they alter cerebral blood flow in normal animals [Foley et al., 1994; McAuley et al., 1994; present thesis]. However, topical application of endothelin antagonists increases the calibre of cortical arterioles *in vivo* in the ischaemic penumbra following a focal cerebral ischaemic challenge [present thesis].

The potent vasoconstrictor effects of the endothelins has attracted much speculation for their involvement in almost every vascular pathology although the precise definition of their pathophysiologic role has proven elusive [Brunner et al., 1992; Ziv et al., 1992; Gellai et al., 1994; Rubanyi & Polokoff, 1994]. The

perivascular microapplication of Bosentan or PD155080 on non-ischaemic feline pial arterioles had minimal effect on arteriolar calibre [Patel et al., 1994, 1995b]. These observations indicate that under normal physiologic conditions there is minimal endothelin mediated vascular tone. The present data suggest that following focal cerebral ischaemia, endogenous endothelins constrict and restrict the dilatation of cortical arterioles in the ischaemic penumbra. The constrictor effects of endogenous endothelins (defined by reversal with the endothelin antagonists, Bosentan and PD155080) are demonstrable early in the evolution of ischaemic injury, at times when neuroprotective drugs, such as NMDA receptor antagonists, can still salvage tissue from irreversible damage [Ozyurt et al., 1988]. The perivascular microapplication of the endothelin receptor antagonist, PD155080 and Bosentan provided an insight not only to a possible pathophysiologic role for endothelins in cerebral ischaemia but also provides a basis for the use of endothelin antagonists in the treatment of occlusive stroke.

A marked reduction in cerebrovascular resistance and the dilatation of the cerebral vasculature occur at the onset of cerebral ischaemia in an attempt to maintain tissue oxygenation. The mechanism for this event has long been attributed to the release of "chemical products of cerebral metabolism" with hydrogen ions, potassium ions and adenosine among the most favoured candidates. In contrast, there is evidence that endogenous constrictor mechanisms evolve, which effectively limit tissue perfusion to ischaemic and peri-ischaemic brain regions [Teasdale et al., 1981]. In focal ischaemia, vasodilator agents (calcium entry blockers, papaverine etc.) have been utilised to produce further decreases in vascular resistance, increase calibre of arterioles and, if systemic hypotension is avoided, increases in tissue blood flow to the ischaemic penumbra [Brandt et al., 1983; Date & Hossmann, 1984; Mohammed et al., 1985].

Several mechanisms have been proposed (and disputed) to restrict tissue perfusion in cerebral ischaemia, including intraluminal obstruction, mechanical compression of the intraparenchymal vessels (consequent to astrocytic swelling and ultimately tissue oedema] and active vasoconstriction [Teasdale et al., 1981; Hatashita

& Hoff, 1986; Wahl & Schilling, 1993). The demonstration that arteriolar calibre in the ischaemic penumbra is increased after microapplication of the endothelin receptor antagonists Bosentan and PD155080, indicates that endogenous endothelins may be involved in limiting blood flow to this zone. While this report clearly demonstrates that there is an endothelin mediated component to alterations in vessel calibre following a focal ischaemic insult, endothelin antagonists (including Bosentan) have been shown to markedly reverse the vasospasm of arteries associated with subarachnoid haemorrhage in several species [Rubanyi & Polokoff, 1994]. In this instance, the stimulus for the increased levels of ET's is likely to be blood products and agents present at the site of injury [Yanagisawa et al., 1988; Kurihara et al., 1989; Ohlstein & Storer, 1992].

The interpretation of the present study depends upon the selectivity of action of Bosentan and PD155080 on endothelin receptors. Bosentan inhibits the binding of radiolabelled ET-1 to endothelin ET_A and endothelin ET_B receptors with K_i values of 5 nM and 95 nM respectively [Clozel et al., 1994]. Bosentan has no demonstrable effects on the binding of other peptides with the exception of Neurokinin A, the binding of which was reduced at very high Bosentan concentrations (10 μM) [Clozel et al., 1994]. In contrast PD155080 inhibits binding of radiolabelled ET-1 to endothelin ET_A and endothelin ET_B receptors with IC_{50}'s of 7.8 nM and 3.5 μM respectively [Doherty et al., 1995]. In feline pial arterioles *in situ*, perivascular microapplication of Bosentan or PD155080 have no effect on arteriolar calibre under normoxic, normocapnic and normotensive conditions [present thesis]. Co-administration of Bosentan or PD155080 with ET-1 (10 nM) inhibits the ET-1-induced constriction of pial arterioles (but not that produced by other vasoconstrictors) with an IC_{50} of 1 μM (for both antagonists) and complete blockade was achieved with co-application of higher concentrations of the antagonists (micropipette concentration = 30 μM) [present thesis]. Despite the slow dissociation of endothelin from their receptors [Marsault et al., 1993; Wu Wong, 1994 & 1995], the present data in normal cats indicates that partial reversal of ET-1-induced cerebral vasoconstriction can be achieved by delayed treatment with either Bosentan or PD155080.

We examined the effects of a combined endothelin ET_A/ET_B receptor antagonist (Bosentan) and an endothelin ET_A selective receptor antagonist (PD155080) on ischaemic arterioles. Bosentan and PD155080 elicited similar magnitude of effects on ischaemic dilated and constricted arterioles. Activation of endothelin ET_A and endothelin ET_B can each mediate vascular contractions depending on the vessel examined. In feline pial arterioles, however, the ET-1-induced vasoconstriction appears to be mediated via endothelin ET_A receptors whereas endothelin ET_B activation produces vasodilatation [Kobari et al., 1994; present thesis]. Receptor characterisation of ET-1 cerebrovascular actions in other species generally supports such a view of the function of ET receptor subtypes [Kitazono et al., 1993; Feger et al., 1994]. The use of a mixed antagonist such as Bosentan does not allow elucidation of the endothelin receptor subtype which mediates the observed cerebrovascular constriction. However, the ability of the endothelin ET_A receptor selective antagonist PD155080 to increase the calibre of both post-occlusion dilated and constricted arterioles indicates that endothelin ET_A receptor activation is probably the dominant mediator of the cerebrovascular effects of endogenous ET-1 following focal ischaemia. The ability of an endothelin ET_A receptor antagonist to increase cerebral blood flow to the ischaemic penumbra and reduce ischaemic neuronal damage has been demonstrated [present thesis]. An important aspect of the pharmacology of Bosentan and PD155080 is their ability to gain access to the vascular smooth muscle of cerebral resistance vessels after intravenous administration [present thesis]. These observations and the demonstration that the calibre of vessels in the ischaemic penumbra can be increased by Bosentan and PD155080 provided a basis for assessing the therapeutic utility of endothelin receptor antagonists in focal cerebral ischaemia.

Recent studies have examined the effects of peptide and non-peptide endothelin receptor antagonists in focal cerebral ischaemia [McAuley et al., 1994; Barone et al., 1995; Checkley et al., 1995; Patel et al., 1995; Umemura et al., 1995] (see Table 15). In the normotensive rat, the combined endothelin ET_A/ET_B receptor antagonist Bosentan and endothelin ET_A selective antagonist BQ-123 elicited

minimal alterations on the volume of ischaemic damage or cerebral blood flow following permanent focal ischaemia [McAuley et al., 1994; Checkley et al., 1995] (see Table 15). In the spontaneously hypertensive rat (SHR) SB217242 and BQ-123 demonstrated 30% and 25% reductions in the volume of ischaemic damage respectively following permanent focal ischaemia [Barone et al., 1995; Patel et al., 1995]. The inability of the Bosentan to significantly alter the volume of ischaemic damage or cerebral blood flow in the rat is probably due to the selectivity of the antagonist for both constrictor endothelin ET_A receptors and dilator endothelin ET_B receptors and the loss of endothelin ET_B receptor mediated increases in cerebral blood flow [McAuley et al., 1994]. The inability of BQ-123 to alter the volume of ischaemic damage in the normotensive strain is probably due to the peptide antagonists not penetrating the blood-brain barrier. In the SHR strain, the ability of BQ-123 to reduce the volume of ischaemic damage is probably due to the damage to the blood-brain barrier n this strain and this allows the peptide antagonist to gain access to the cerebrovasculature [Horie et al., 1978]. The combined endothelin ET_A/ET_B receptor antagonist SB217242 reduced the volume of ischaemic damage in a rat model of focal cerebral ischaemia [Barone et al., 1995]. However, the poor blood-brain barrier permeability of this compound required 1 week of pre-dosing. The combination of tissue Plasminogen activator (t-PA) and FR139317 did not the increase the protective effect of t-PA in a thrombo-embolic model in the rat [Umemura et al., 1995].

Reports of increased plasma endothelin levels in patients with acute ischaemic stroke has facilitated interest in the therapeutic potential of endothelin receptor antagonists in the treatment of stroke [Ziv et al., 1992; Estrada et al., 1994]. The elevations in plasma endothelin-1 levels ranged from 4 to 28 pg/ml in the first 72 hours following an ischaemic stroke (control levels 2 pg/ml) [Ziv et al., 1992; Estrada et al., 1994], however, the pathological significance of elevations in plasma endothelin-1 levels in the progression of cerebral ischaemic damage is unclear since endothelin-1 does not cross the blood-brain barrier and the evidence indicates that the actions of the endothelins are paracrine in nature [Yoshimoto et al., 1990; Wagner et al., 1992; Mima et al., 1989]. Increases in tissue endothelin immunoreactivity have

been demonstrated in experimental focal cerebral ischaemia (permanent and transient models) (magnitude 50-80% increases from controls) [Barone et al., 1994; Duverger et al., 1992; Viossat et al., 1993]. The increases in tissue endothelin immunoreactivities have been measured at 4 hours (50% increase compared to sham animals) and with marked elevations at 24 and 72 hours following focal cerebral ischaemia (400% increase compared to sham animals at 72 hours) [Barone et al., 1994; Duverger et al., 1992; Viossat et al., 1993].

Endothelin receptor antagonists have no significant effect on cerebral arterial or pial arteriolar calibre under non-pathologic conditions *in vivo* nor do they alter cerebral blood flow in normal animals [Foley et al., 1994; McAuley et al., 1994; present thesis]. Following the demonstration that the topical application of endothelin antagonists increases the calibre of cortical arterioles *in vivo* in the ischaemic penumbra following a focal cerebral ischaemic challenge [Patel et al., in press], we proceeded to examine the effects of the endothelin ET_A receptor antagonist PD156707 on peri-ischaemic cerebral blood flow (measured by laser doppler flowmetry) and on neuronal ischaemic damage.

Despite the existence of multiple homeostatic mechanisms to protect blood flow to the brain from compromise, constrictor mechanisms which limit tissue perfusion to the ischaemic penumbra following cerebral ischaemia are well recognised [Hossmann et al., 1973]. A variety of mechanisms for reductions in CBF following focal ischaemia have been proposed, these include vasoconstriction, increased tissue pressure and luminal obstruction [Teasdale et al., 1981; Hatashita & Hoff, 1986; Wahl & Schilling, 1993]. However, the precise mechanism for the maintenance of reduced post-ischaemic cerebral blood flow is unknown. The ability of the endothelin receptor antagonist PD156707 to increase cerebral perfusion indicates a role for endothelin in the regulation of vascular tone and cerebral blood flow following MCA occlusion. The sources and mechanisms that underlie the increases in endothelin mediated vascular tone are presently unclear but increases in tissue and CSF endothelin immunoreactivities following focal cerebral ischaemia have been reported by a

number of groups [Ziv et al., 1992; Viossat et al., 1993; Barone et al., 1994] [see following section].

PD156707 *per se* failed to alter the calibre of non-ischaemic feline pial arterioles indicating that, under the experimental conditions used, there is minimal endothelin mediated tone in cerebral resistance vessels [Patel et al., in press]. The immediate response following MCA occlusion is a decrease in cerebrovascular resistance in order to compensate for the reduction in blood flow. A number of investigations have been performed where vasodilator substances, such as papaverine and nimodipine, increase cerebral blood flow provided systemic hypotension is avoided [Kazda et al., 1982; Brandt et al., 1983; Date & Hossmann, 1984; Mohammed et al., 1985]. The perivascular microapplication of the calcium channel antagonist nifedipine demonstrated an increase in calibre of post-ischaemic constricted arterioles indicating an active vasoconstriction of these blood vessels [Brandt et al., 1983]. The perivascular microapplication of endothelin receptor antagonists on ischaemic arterioles has demonstrated an increase in endothelin mediated vascular tone following focal ischaemia [present thesis].

Although the role of the endothelins as neurotransmitters or neuromodulators has been suggested, the effects of endothelin receptor antagonists *per se* on neuronal function has not been examined [Gross et al., 1993; Berrino et al., 1994]. There are very few studies examining the efficacy of endothelin receptor antagonists acutely following focal cerebral ischaemia [McAuley et al., 1994; Patel et al., 1995; Patel et al., in press]. PD156707 (at a single dose validated for efficacy) reduces the volume of focal ischaemic damage, however, the mechanism of action for this reduction in unclear [Figure 45]. It can be speculated that since PD156707 increases perfusion in the peri-ischaemic regions the effect may be the result of an enhancement of peri-ischaemic cerebral perfusion [Figure 46].

The endothelin receptor antagonists targeted at cerebral resistance blood vessels must gain access to the adventitial surface of cerebral resistance arterioles [Edvinsson et al., 1993; present thesis]. The intravenous dose of PD156707 examined in this investigation attenuates exogenous ET-1 induced vasoconstriction of non-

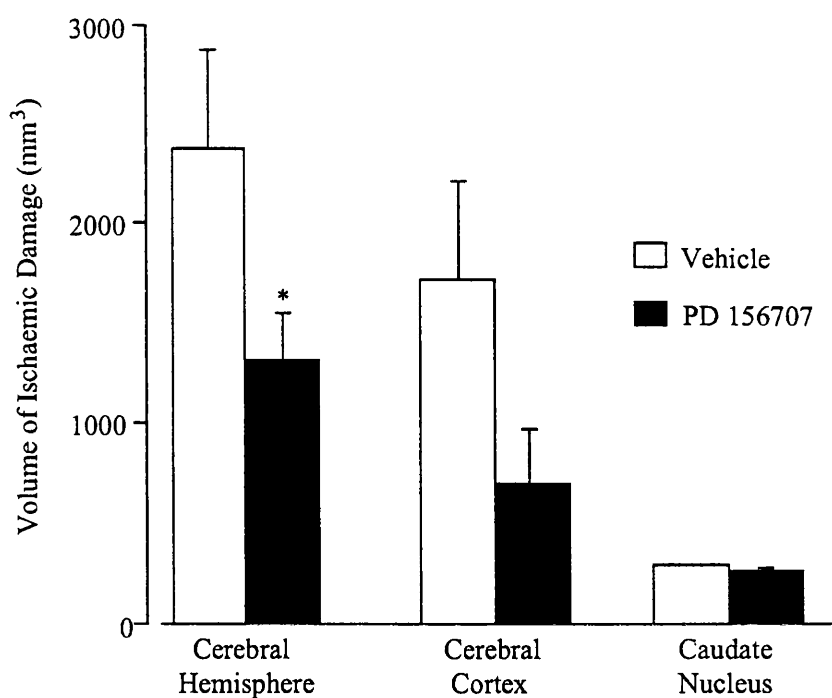


Figure 45 Effect of PD156707 (3 μmol/kg bolus + 5 μmol/kg/hr infusion) upon volume of ischaemic damage after permanent MCA occlusion. PD156707 was administered 30 min following MCA occlusion. Data are expressed as mean ± S.E.M (n = 5 in each group). *$P<0.05$ compared to vehicle treated animals (one tailed Student's t-test).

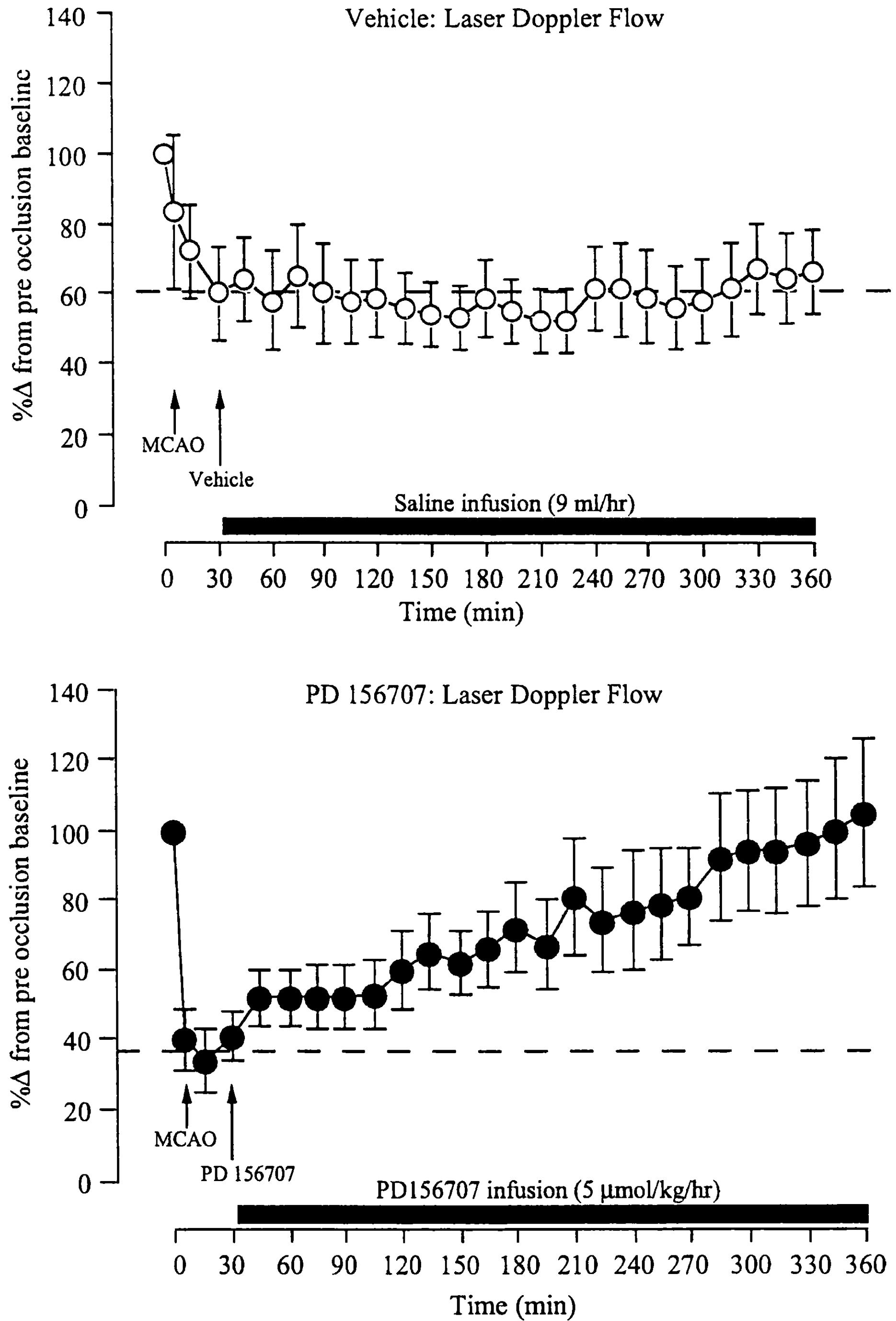


Figure 46 Cerebral Perfusion (determined by laser doppler flowmetry) in vehicle and PD156707 treated animals. Data are mean ± S.E.M from 8-10 probes overlying the ectosylvian and suprasylvian gyri in 10 cats (5 in each group).

The vehicle (saline) was administered 30 min. following MCA occlusion as an intravenous bolus injection followed by a continuous infusion (top panel).

PD156707 (3 µmol/kg) was administered as an intravenous bolus injection 30 min. after MCA occlusion followed by a continuous intravenous infusion (5 µmol/kg/hr) (lower panel).

ischaemic arterioles demonstrating that bioactive amounts of PD156707 gain access to the adventitial surface of cerebral resistance arterioles. Our unpublished observations have indicated that approximately 12% of the plasma drug level is detectable in the CSF [T. R. Patel, unpublished observations]. This validated dose of PD156707 was subsequently used in the focal ischaemia investigations and demonstrated its ability to increase cerebral perfusion and reduce ischaemic damage.

PD156707 is a highly selective antagonist for endothelin receptors. PD156707 in concentrations up to 10 mM did not inhibit the binding of 25 ligands to various neurotransmitter systems including angiotensin II, neuropeptide Y and vasoactive intestinal peptide [Reynolds et al., 1995]. *In vitro* pharmacological data for PD156707 indicates that this compound is selective for the endothelin ET_A receptor subtype with an IC_{50} 0.3 nM and 420 nM at endothelin ET_A and endothelin ET_B receptors respectively [Doherty et al., 1995]. In *in vitro* functional studies PD156707 demonstrates a 100 fold selectivity for the endothelin ET_A receptor compared to the endothelin ET_B receptor [Reynolds et al., 1995]. The dose of PD156707 used in the present investigations markedly attenuates the vasoconstrictor response to ET-1 (1 nmol/kg, intravenous bolus) and does not affect the vasodilator response (endothelin ET_B receptor mediated) elicited by this dose of ET-1 indicating the selectivity of PD156707 for the endothelin ET_A receptor [T. R. Patel, unpublished observations].

Previous investigations to characterise the endothelin receptor subtypes in feline cerebral arterioles has demonstrated that endothelin ET_A receptors mediate vasoconstriction and endothelin ET_B receptors mediate vasodilatation [Kobari et al., 1994; present thesis]. The increase in cerebral perfusion in peri-ischaemic areas induced by PD156707 is probably due to antagonism of endothelin ET_A receptor mediated vasoconstrictions and, perhaps, the unmasking of endothelin ET_B receptor mediated vasodilatation [Kobari et al., 1994; present thesis]. The contribution of endothelin ET_B receptors to the regulation of perfusion in cerebral ischaemia could be assessed by the selective endothelin ET_B receptor antagonists and agonists. The ability of the endothelin receptor antagonists to increase the calibre of cerebral resistance arterioles, increase cerebral perfusion in peri-ischaemic areas and reduces hemispheric

ischaemic damage after experimental focal cerebral ischaemia, indicates the importance of endogenous endothelins in the regulation of cerebrovascular tone in the post-ischaemic phase and the therapeutic potential of endothelin receptor antagonists in the amelioration of ischaemic damage.

Critical appraisal of techniques used for measuring cerebral blood flow

The principles for the measurement of cerebral blood flow were first described by Kety and Schmidt in the 1940's. The techniques were based upon the exchange of inert gases, such as nitrous oxide, between the blood and the brain tissue. The original techniques were modified to measure the cerebral blood flow using radioactive tracers by Sokoloff and colleagues and the developments of polarographic techniques to measure cerebral blood flow using inert gases such as hydrogen.

Hydrogen Clearance

The hydrogen clearance technique for the measurement of cerebral blood flow was devised by Aukland and colleagues in the 1960's. However, prior to this Misrahy and Clark (1956) had demonstrated that electrical potentials measured using platinum electrodes implanted the cerebral cortex were altered when the inspired gases contained hydrogen. The electrical potentials increased in magnitude when CO2 was inhaled and decreased when barbiturates were administered [Misrahy & Clark, 1956]. The original techniques described were selective for hydrogen but there was no correlation between the hydrogen concentration and the electrical potential generated when using platinum electrodes. The studies of Hyman (1961) and Aukland et al. (1964) correlated the hydrogen concentration in the tissue with the electrical potential generated.

There are a number of properties of hydrogen that led to the use of the gas as tracer for the measurement of cerebral blood flow. Molecular hydrogen is not normally found in tissues and it is freely diffusible across lipid membranes in biological tissues. additionally, hydrogen possess a low water/gas partition coefficient and thus when the gases are turned off it is readily removed from the circulation via the lungs.

The hydrogen clearance technique used in the present thesis was a polarographic technique where an electrode is implanted into the tissue of interest. In the present studies platinum electrodes were used (See section 2.3 for methods). The polarographic technique is based on the principle that the molecular hydrogen is

converted to hydrogen ions at the polarised platinum electrodes implanted into the brain. The resultant electrical signal generated is recorded on to a chart recorder and the cerebral blood flow is calculated by the fitting of the data to an exponential function and from the equation given in section 2.3. In brain tissues the process of ionisation of the hydrogen is limited by the rate of diffusion of the hydrogen to the electrode and since the diffusion coefficient of hydrogen is one, the ionisation is limited by the cerebral blood flow to the area where the platinum electrode is implanted [Doyle et al., 1975; Young, 1980].

The hydrogen clearance technique has number of advantages for the measurement of blood flow. Cerebral blood flow can be measured from any site in the brain where an electrode can be placed. The placement of multiple electrodes in various brain regions would permit the examination of cerebral blood flow across the brain. The electrodes are normally positioned at a particular site for the duration of the experimental period and this allows the multiple determination of cerebral blood flow and a temporal profile of the cerebral blood flow can be examined in the brain region of interest. The hydrogen is normally introduced by inhalation and the alteration of the inspired gas mixture and so is relatively easy to execute the procedure. The hydrogen clearance technique has been used for the measurement of cerebral blood flow in a range of species under normal and ischaemic conditions [Fieschi et al., 1969; Gotoh et al., 1966; von Kummer & Herold, 1986, von Kummer et al., 1986; Symon et al., 1970; Symon et al., 1976; Haining et al., 1968; Mackay et al., 1996; Young, 1980].

There are a number of limitations to the use of hydrogen clearance to measure cerebral blood flow. The electrodes used for the measurement of CBF are normally implanted into the brain regions of interest. Local tissue injury resulting from the placement of the electrodes may alter the cerebral blood flow in the vicinity of the electrode [Young et al., 1980]. The clearance of hydrogen from the brain tissue is polyexponential in nature and the calculation of cerebral blood flow from the monoexponential curves normally used will give rise to erroneous CBF measurements [Doyle et al., 1975; Young, 1980; von Kummer & Herold, 1986]. A major assumption of the hydrogen clearance technique is that the arterial concentration of the gas falls to

zero once the gas has been turned off [Young, 1980]. There is evidence that in the first minute following the turning off of the gas, there is a recirculation of hydrogen through the small arteries and arterioles [Stosseck, 1970]. Fieschi et al. (1969) demonstrated that in the major arteries 95% of the hydrogen is removed from the circulation in the first minute. However, in the smaller arteries and arterioles the clearance of hydrogen lags behind by a few minutes due to the recirculation into the microvasculature from the tissue [Stosseck et al., 1970]. The electrodes used for the measurement of hydrogen are polarised to a set voltage (+700 mV). The electrodes used can also be sensitive to the oxidation of oxygen at the electrodes.

The limitations of the hydrogen clearance techniques are overcome by a variety of methods. The original studies of Aukland and colleagues used electrodes of approximately 1 mm diameter but the electrodes used in more recent studies are of much smaller diameter, typically <250 μm. The smaller electrode diameters result in less localised tissue damage although due to the invasive nature of the implantation tissue damage cannot be completely avoided. The recirculation artefacts that occur following the removal of the hydrogen are counteracted by the discarding of the first minute of the clearance curve. At the end of the clearance period a shift in the baseline of the curve is normally observed. The reasons for the shift are unclear but its has been suggested that the baseline shift may be the result of the oxidation of substances such as ascorbate following the induction of cerebral ischaemia (oxidation occurs at levels close to +600 mV). The baseline shift was carefully monitored during the course of the experiment and at the end of each flow determination the baselines were reset to the pre-occlusion baselines. The oxidation of oxygen at the electrode site resulting in a contamination of the hydrogen signal is counteracted by the polarising the platinum electrode to greater than + 600 mV and the contribution of oxygen to the signal is negligible at this voltage [Kissinger et al, 1973; Young, 1980]. It has been observed that the signal at electrodes polarised to +200 mV is contaminated by the oxidation of catecholamines, haemoglobin and oxygen in addition to the oxidation of hydrogen [Kissinger et al, 1973; Young, 1980]. The hydrogen clearance technique has been used in investigations in cerebral ischaemia. During the measurement of cerebral blood

flow the diffusion of the hydrogen is assumed to be constant. Under ischaemic conditions, the development of cerebral oedema will alter the diffusion of hydrogen in the tissue and thus have an adverse effect on the measurement of cerebral blood flow. The evidence indicates that there is no significant cerebral oedema at time points less than approximately 6 hours. The use hydrogen clearance in cerebral ischaemia investigations with chronically implanted electrodes may have results to the contrary.

A persistent problem associated with the hydrogen clearance technique for the measurement of cerebral blood flow is the localisation of the site of blood flow and the volume of tissue involved in there cording of the cerebral blood flow. The investigation performed to investigate this have indicated that tissue involved in the estimation of cerebral blood flow is between 2-5 mm from the site of the electrode[von Kummer & Herold, 1986]. Additionally, the disruption of the blood-brain barrier by the hydrogen electrodes increases permeability of drugs that may not normally penetrate the blood-brain barrier. The ability of Bosentan to increase in cerebral blood flow following transient occlusion of the common carotid arteries may be the result of increased concentrations of Bosentan penetrating the blood-brain barrier.

[^{14}C]-Iodoantipyrine autoradiography

The original autoradiographic techniques were designed for the use with radioactive tracers such as trifluoroiodoethane ([^{131}I] CF_3I) and [^{14}C]-Antipyrine [Landau et al., 1955]. The use of radio labelled gases was developed because of the unrestricted diffusion of the gases across the lipid membranes from the blood stream. However, [^{131}I] CF_3I had a relatively short half-life and the problem of storage. The technique was modified by Reivich and colleagues (1969) to allow the use of [^{14}C] labelled Antipyrine. The use of [^{14}C] labelled Antipyrine had the added the benefit of the long half life compared to the iodinated ligands but the measurements of cerebral blood flow were underestimated when [^{14}C]-Antipyrine was used. [^{14}C]- Antipyrine affected the measurements of cerebral blood flow because of its ability to diffuse across the blood-brain barrier. The use of [^{14}C]-Antipyrine was superseeded by the

use of [^{14}C]-Iodoantipyrine which had a higher partition coefficient (lipid/water) and the measurements of cerebral blood flow were similar to the values obtained by CF_3I [Sakurada et al., 1978].

The use of Iodoantipyrine autoradiographic has a number of advantages. The radiolabelled tracer is a freely diffusible tracer and hence the measurements of cerebral blood flow in the region of interest is limited by the level of blood flow. The autoradiography technique has excellent spatial resolution and permits the measurement of CBF in multiple brain regions. Additionally, the brain areas with alterations in patterns of blood flow can be visualised on the autoradiograms and determined. In comparison, the hydrogen clearance relies on the pre-selection of the brain region of interest and a limited number of sites can be examined at any one point. The autoradiographic technique also has the added advantage that since extensive studies have been carried out on the neuronal pathways in the brain we can determine the patterns of cerebral blood flow in the areas following interventions. An important advantage of the Iodoantipyrine autoradiography technique is that it is non-invasive. Unlike the hydrogen clearance technique where the platinum electrodes are implanted in the brain tissue, the radiolabelled tracer is administered intravenously. The implantation of the hydrogen electrodes can lead to localised tissue damage and may lead to aberrant readings in the values of cerebral blood flow.

One of the main disadvantages of autoradiographic techniques for measuring cerebral blood flow is that in order to gain temporal insight into the alterations in cerebral blood flow multiple groups would be required for each individual time point to be investigated. The methodology for the Iodoantipyrine technique is complex and requires the accurate measurement of parameters such as the time of the blood sample, and the duration of the experiment [Sakurada et al., 1978; Patlak et al., 1984]. The inaccurate measurement of any of these parameters can result in marked errors in the value of cerebral blood flow obtained [Patlak et al., 1984]. The use of radioactive tracers, such as Iodoantipyrine, also have the additional problems of the back flux of the tracer from the tissue into the blood [Patlak et al., 1984]. The errors arising from the potential back flux of the Iodoantipyrine are reduced by using a short experimental

schedule (30 s) infusion and the use of a ramped infusion schedule, with the arterial concentration of the Iodoantipyrine tracer increasing with time [Patlak et al., 1984].

The benefits of Iodoantipyrine autoradiography are the most marked when used to examine the alteration in cerebral blood flow following focal and cerebral ischaemia. The technique is commonly used in conjunction with potential neuroprotective drugs and to gain insight into their mechanism of action. The complexity and the cost of conducting investigations using Iodoantipyrine has seen the increased use of laser doppler flowmetry in cerebral blood flow investigations. However, laser doppler flowmetry measures the velocity of the erythrocytes in cerebral tissue rather than the flow of plasma and the erythrocytes and thus cerebral perfusion is measured in arbitrary units. Laser doppler flowmetry has excellent on-line and temporal monitoring of the alterations in cerebral perfusion but poor spatial resolution (similar to hydrogen clearance) [Fabricius & Lauritsen, 1994]. The laser doppler probes are normally placed on the surface of an intact dura and used to monitor the alterations in cortical perfusion. Preliminary evidence has compared the values of CBF obtained by using Iodoantipyrine autoradiography and laser doppler flowmetry [Fabricius & Lauritsen, 1995]. The results indicated that laser doppler flowmetry overestimated the alterations in cerebral perfusion when compared to CBF obtained by Iodoantipyrine autoradiography. The measurement of erythrocyte flow by the laser doppler technique and total CBF by the autoradiography technique is one possible explanation for the discrepancies in the two techniques.

4.2.2 *Sources and Triggers for endothelin synthesis following cerebrovascular injury*

The sources of endothelin release following cerebrovascular injuries are unclear. A number of triggers for the synthesis of endothelin peptides from astrocytes, neurones or endothelial cells have been suggested [MacCumber et al., 1990; Ehrenreich et al., 1991] and these include tissue hypoxia, thrombin, transforming growth factor-ß (TGF-ß), oxyhaemoglobin and mechanical shear stress [Yanagisawa et al., 1988; Kurihara et al., 1989; Malek & Izumo, 1992; Ohlstein & Storer, 1992; Tippler et al., 1994; Kourembanas et al., 1993; Elton et al., 1992; Kuchan & Frangos, 1993; MacArthur et al., 1994; Morita et al., 1993; Ehrenreich et al., 1990] (Table 17).

Thrombin is an important component of the coagulation cascade. Thrombin has been shown to induce an increase in ET-1 mRNA levels and ET-1 release [Kurihara et al., 1989; Fukunaga et al., 1991; Zoja et al., 1991]. However, exposure of renal mesangial cells to thrombin results in decrease in endothelin receptor numbers [Albrightson et al., 1995]. The endothelins have been implicated in the pathogenesis of vasospasm following subarachnoid haemorrhage. Subarachnoid haemorrhage is often associated with the rupture of cerebral blood vessels and the presence of thrombin in the subarachnoid space may increase the synthesis and release of endothelin in the vicinity of the haemorrhage.

Oxyhaemoglobin has been implicated as a pathogen for development of vasospasm following subarachnoid haemorrhage. Oxyhaemoglobin stimulates the release of ET-1 from endothelial cell cultures [Ohlstein & Storer, 1992]. Lysed erythrocytes (from human and rat) facilitate the production of ET-1 from the precursor, pre pro- ET-1 [Tippler et al., 1994]. Platelets have been demonstrated to increase ET-1 mRNA [Ohlstein et al., 1991] and potentiate the release of ET-1 from endothelial cell cultures [Ohlstein & Storer, 1992; Umekawa et al., 1994]. Platelet derived growth factor (PDGF) also facilitates the release of ET-1 from endothelial cells [Janat et al., 1992; Kohno et al., 1993]. In cerebral ischaemia an increased

Table 17

Local triggers for the release of endothelins

Triggers	References
Platelet derived growth factor (PDGF)	Bakris et al., 1991; Janat & Liaw, 1992; Kohno et al., 1993
Lysed erythrocytes	Tippler et al., 1994
Platelets	Ohlstein et al., 1991; Ohlstein & Storer, 1992; Umekawa et al., 1994
Tissue hypoxia	Ehrenreich et al., 1990; Morita et al., 1993; Kourembanas et al., 1993;
Thrombin	Yanagisawa et al., 1988; Kurihara et al., 1989
Transforming growth factor-ß (TGF-ß)	Yanagisawa et al., 1988; Kurihara et al., 1989
Oxyhaemoglobin	Ohlstein & Storer, 1992; Tippler et al., 1994
Mechanical shear stress	Elton et al., 1992; Malek & Izumo, 1992; Kuchan & Frangos et al., 1993

expression of PDGF has been observed [Iihara et al., 1994] and there is the potential of interactions between PDGF and ET-1 in the development is ischaemic damage.

Aggregated platelets release the platelet derived polypeptide TGF-ß and TGF-ß can be synthesised in vascular endothelial cells. TGF-ß has been shown to increase ET-1 mRNA levels in endothelial cells [Kurihara et al., 1989; Murata et al., 1995]. Increased release of TGF-ß has been observed in myocardial infarction and the TGF-ß may be produced from the platelets, vascular endothelial cells or vascular smooth muscle cells. The role of TGF-ß in the development of vascular injury may be enhanced by its ability to increase the synthesis and release of ET-1 [Casscells et al., 1990; Brown et al., 1991].

Thus the implication is that blood *per se* may not result in the vasoconstrictive response but constituents contained in blood, such as the platelets, may cause the generation of endothelins through the induction of endothelin mRNA and may indirectly result in the production of ET-1 and the manifestation of delayed vasospasm.

There are a number of possible triggers for the release and synthesis of endothelins including tissue hypoxia [Elton et al., 1992]. The reduction in cerebral blood flow and the possibility of blood pooling within the ischaemic region could trigger the release or synthesis of endothelins [Kurihara et al., 1989; Ohlstein & Storer, 1992]. Products from platelets such as TGF-ß and PDGF are known inducers of endothelin synthesis and may also provide a trigger for the release/synthesis of endothelins following an ischaemic insult [Kurihara et al., 1989]. Hypoxia, following an occlusive insult, is the most likely candidate as the trigger which elicits an increase in endothelins to vasoactive concentrations. Several studies in cell culture would support this idea [Elton et al., 1992]. However, evidence in the present studies indicate that rheologic factors (or reactive alterations in vessel calibre) *per se* are insufficient to elicit vasoactive amounts of endothelins, since there was an absence of a vasomotor response to Bosentan or PD155080 in the post-occlusion dilated vessels overlying the parasagittal gyrus (blood flow derived from the anterior cerebral artery) following MCA occlusion in the cat.

Among the many features associated with cerebrovascular disease is damage to the vascular endothelium either due to mechanical damage by clots, increased mechanical or fluid shear stress or disruption of the endothelial cell membranes by free radicals produced during cerebral ischaemia [Siesjo et al., 1992a; Phillis, 1994]. An increase in fluid shear stress on endothelial cells, e.g. experienced in recanalisation of occluded vessels, has been shown to stimulate increases in endothelin mRNA and endothelin release [Kuchan & Frangos, 1993]. Blood in the intracranial space or damage to the vascular endothelium can increase the ability of platelets to aggregate and increases leucocyte adhesion to endothelial cells [Grogaard et al., 1989; Dirnagl et al., 1994]. Following subarachnoid haemorrhage constituents of blood such as platelets can induce the release of vasoconstrictor factors such as TGF-ß and increase the production of ET-1 [Kurihara et al., 1989]. The increased expression of ET-1 mRNA or the release of ET-1 may be involved in the development of delayed vasospasm following subarachnoid haemorrhage. In astrocytic cell cultures, ET-1 has been demonstrated to evoke the release of arachidonic acid [Tence et al., 1992]. The role of arachidonic acid metabolites in the generation of free radicals has been a mechanistic basis for the progression of neuronal damage following cerebral ischaemia [Phillis, 1994]. In cerebral ischaemia, hypoxia within the ischaemic territory may stimulate the production of ET-1 and have a role in the progression of ischaemic neuronal damage.

Evidence from cell culture studies indicate that shear stress may induce an increase in ET-1 mRNA or ET-1 release [Kuchan & Frangos et al., 1993; Morita et al., 1993; MacArthur et al., 1994]. Differences in the source and target of ET's may occur depending on the brain injury [Kurihara et al., 1989; Barone et al., 1994].

Apart from the proposed cerebrovascular role of the endothelin peptides, evidence from binding assays has shown the presence of binding sites for endothelins in both rodent and human brain [Ambar et al., 1989; Jones et al., 1989; Koseki et al., 1989] and spinal cord [Niwa et al., 1992] and glia [Koseki et al., 1989; MacCumber et al., 1990; Ehrenreich et al., 1991]. Glial cells may be an important source of endothelins within the CNS [MacCumber et al., 1990; Marsault et al., 1990; Ehrenreich et al., 1991]. However, these are not the only sources of production or

binding sites within the brain. Endothelial cells and vascular smooth muscle cells of cerebral vessels have also shown the ability to generate endothelins and the existence of binding sites has been demonstrated in these cells [Yoshimoto et al., 1990; Resink et al., 1990; Hirata et al., 1993; Takenaka et al., 1993]. The endothelin receptor subtype(s) mediating endothelin actions within the CNS are unclear. The mixed action of Bosentan at endothelin ET_A and endothelin ET_B receptors could be harnessed to investigate other roles of endothelin within the CNS. The trigger for and sources of the putative increased perivascular endothelins in focal ischaemia are presently unclear. Intracellular concentrations of ET-1, as determined by radioimmunoassay, are increased 24 hours after a focal (MCA occlusion) or global (two vessel occlusion plus hypotension) ischaemic insult [Duverger et al., 1992; Barone et al., 1994]. Endothelial cells and neurones from the brain and spinal cord express ET mRNA and ET-immunoreactivity, implicating these cells as potential sources of endothelins following an ischaemic insult [Giaid et al., 1989; Lee et al., 1990; MacCumber et al., 1990]. Although secretory granules are not generally found in endothelial cells, in contrast to neurones, it is known that endothelial cells do contain some ET-like peptide which may be released immediately under certain conditions [MacArthur et al., 1994]. This supports evidence that *de novo* synthesis of this peptide (which may take hours) is not always necessary and may explain the ability of Bosentan and PD155080 in the present study, to alter or reverse ischaemically-induced perturbations in pial vessel calibre relatively acutely after the insult.

The cellular source of endothelins following a cerebrovascular insult is unclear. The presence of endothelin mRNA and release of ET-1 from cerebrovascular endothelial cells have been demonstrated [Yoshimoto et al., 1990; Saito et al., 1991]. In brain tissue, both astrocytes and neurones have been reported to possess endothelin mRNA [MacCumber et al., 1990; Ehrenreich et al., 1991]. The release of endothelin from endothelial cells or from neurones and astrocytes may account for the rapid perturbations in blood vessel calibre following MCA occlusion [MacArthur et al., 1994]. The presence of stores of endothelins in some cells indicates that *de novo* synthesis of endothelins may not be necessary for the development of the acute effects.

4.2.3 Pharmacotherapy of cerebral ischaemia: comparisons with endothelin receptor antagonists

Pharmacotherapy of cerebral ischaemia has two general approaches. Firstly, pharmacological interventions are directed at various points in the cascade of neurochemical events which lead to neuronal damage, for example, NMDA and AMPA receptor antagonists, glutamate release inhibitors, nitric oxide synthase inhibitors, free radical scavengers and the inhibition of the effects of the cytokines [Rothwell & Relton, 1993; Gill, 1994; Muir & Lees, 1994; Phillis, 1994]. Secondly, pharmacological interventions that are directed at removing the primary intraluminal obstruction (e.g. Tissue Plasminogen Activator) or by reducing the vasoconstrictor influences on collateral vessels to the tissue at risk (e.g. L-type calcium channel antagonists) [Kazda et al., 1982; Fisher & Grotta, 1993; Overgaard, 1994]. The demonstration of elevated endothelin levels following subarachnoid haemorrhage, focal and global cerebral ischaemia has led to the investigation of endothelin receptor antagonists as potential therapeutic agents.

The ability of endothelin receptor antagonists to reduce ischaemic neuronal damage following focal and global cerebral ischaemia has been demonstrated in a number of recent investigations. However, the precise mechanism of action of the endothelin receptor antagonist is at present unclear. One can speculate that since the endothelin receptor antagonist increases cerebral perfusion and reduce ischaemic neuronal damage following focal cerebral ischaemia, the neuroprotective effects of the antagonist may be the result of increasing cerebral blood flow to the ischaemic penumbra [Patel et al., in press].

Focal Cerebral Ischaemia

The ability of L-type calcium channel antagonists, primarily nimodipine, to reduce ischaemic neuronal damage following focal cerebral ischaemia has been investigated extensively in a variety of species. The results obtained with nimodipine are diverse with some authors demonstrating significant neuroprotection while others

have demonstrated the absence of any significant effects. Previous investigations have demonstrated that the L-type calcium channel antagonist nifedipine increased the calibre of cortical surface arterioles following MCA occlusion. The actions of the endothelin receptor antagonist to similarly increase the calibre of cortical surface arterioles indicated the existence of endothelin mediated vasoconstrictor tone on these vessels. The ability of nimodipine to reduce ischaemic neuronal damage has been attributed to a combination of reduction in neuronal influx of calcium and an increase in cerebral blood flow. In the rat, pre-treatment with nimodipine reduced ischaemic neuronal damage (20 - 30% reduction in hemispheric infarct) following focal cerebral ischaemia [Mohammed et al., 1985; Jacewicz et al., 1990a] while post-treatment failed to demonstrate a reduction in the volume of ischaemic neuronal damage [Gotoh et al., 1986]. The administration of nimodipine offset vasoconstriction observed in the cerebral arterioles following focal cerebral ischaemia and reduced the volume of tissue perfused by cerebral blood flow the ischaemic threshold [Jacewicz et al., 1990b]. Although post-treatment with nimodipine failed to reduce the volume of ischaemic neuronal damage, there was evidence that nimodipine increased the cerebral blood flow in the brain regions on the margins of the territory of the middle cerebral artery. Bosentan and BQ-123 failed to reduce the volume of ischaemic damage following focal cerebral ischaemia in the normotensive rat (see Table 15) [McAuley et al., 1994; Checkley et al., 1995]. Unlike nimodipine, Bosentan failed to demonstrate any significant increases in cerebral blood flow following focal cerebral ischaemia in the rat [McAuley et al., 1994]. In the spontaneously hypertensive rat, BQ-123 and SB217242 reduced the volume of ischaemic neuronal damage following focal cerebral ischaemia [Barone et al., 1995; Patel et al., 1995].

The effects of nimodipine have also been examined following focal cerebral ischaemia in the cat. The intracarotid infusion of nimodipine prior to the ischaemic insult failed to alter the volume of infarct following focal cerebral ischaemia [Barnett et al., 1986] while intravenous pre-treatment reduced the volume of ischaemic neuronal damage and increased the calibre of cortical surface arterioles in the territory of the middle cerebral artery [Mokry et al., 1987; Mokry et al., 1989]. The

administration of nimodipine post-MCAO failed to demonstrate any significant reduction in the volume of ischaemic damage [Mokry et al., 1987]. These observations are in stark contrast to our observations in the cat where the post-treatment with an endothelin receptor antagonist increased the cerebral perfusion and reduced the volume of ischaemic neuronal damage [Patel et al., in press].

The absence of any significant effect observed by either the endothelin receptor antagonist or nimodipine can be attributed to a variety of factors. The differences in the doses of nimodipine and administration schedules of nimodipine could account for the absence of any significant effect in focal cerebral ischaemia. Similar issues regarding the adequacy of drug dosing may account for the absence of any significant effects with endothelin receptor antagonists. However, the selectivity of the endothelin receptor antagonists for dilator endothelin ET_B receptors or constrictor endothelin ET_A receptors is one possible explanation for the absence of any effects (particularly Bosentan). The inability of the peptide endothelin receptor antagonist (BQ-123) to permeate the blood-brain barrier explains the absence of any effect. Other possible explanations include the differences in the cerebrovascular anatomy of the rat and cat [Coyle & Jokelainen, 1982; Mchedlishvili & Kuridze, 1984]. The lower density of connections between collateral vessels in the cerebral cortex of the rat is one possible explanation for the failure of Bosentan to increase cerebral blood flow following focal cerebral ischaemia [Coyle & Jokelainen, 1982; Mchedlishvili & Kuridze, 1984].

The elevations of extracellular glutamate concentrations associated with focal cerebral ischaemia have formed the basis of anti-ischaemic drug development. For the last decade, NMDA and non-NMDA receptor antagonists have been at the forefront of anti-ischaemic drug development. The antagonists that have been developed have been targeted at the NMDA receptor complex and at the AMPA receptors (see section 1.2.2 for pathophysiology of cerebral ischaemia).

The neuroprotective effects of a variety of AMPA and NMDA receptor antagonists have been examined in a range of species. NBQX and LY293558, which are potent AMPA receptor antagonists, have been demonstrated to reduce the volume

of ischaemic neuronal damage following focal cerebral ischaemia in the rat and cat [Bullock et al., 1994; Gill, 1995] The NMDA receptor antagonist are targeted at multiple sites on the NMDA receptor complex including the neurotransmitter recognition site, glycine site, ion channel and the polyamine site (see section 1.2.2 for functional significance of receptor sites). The non-competitive NMDA ion channel blockers (MK-801; CNS1102) have demonstrated marked efficacy in experimental focal cerebral ischaemia. In the feline model of permanent focal cerebral ischaemia, MK-801 reduced the hemispheric volume of ischaemic damage by 50% when administered pre or post-MCAO [Ozyurt et al., 1988; Kurumaji et al., 1989]. The neurotransmitter recognition site antagonist, CGS19755 and D-CPP-ene, also reduce the volume of ischaemic damage following focal cerebral ischaemia in the cat [McCulloch et al., unpublished observations; Chen et al., 1991a]. The pre-treatment with glutamate release inhibitors (CI-977) and low affinity NMDA ion channel blockers (Remacemide) have demonstrated the ability to reduce ischaemic neuronal damage [Mackay et al., 1993; Bannan et al., 1995; Mackay et al., in press]. In the rat, the magnitude of reduction of ischaemic damage is greater with the competitive and non-competitive NMDA receptor antagonists than with the AMPA receptor antagonist. The increased prevalence of NMDA receptors compared to AMPA receptors in the brain may account for the differences in the efficacy of the two classes of compounds. The neuronal damage that is associated with cerebral ischaemia is normally the result of elevated levels of intracellular calcium. The calcium can enter the cell through the ion channels associated with either the AMPA receptors or the NMDA receptors. Since there are more NMDA receptors, the antagonism of the AMPA receptors may have a limited effect on preventing neuronal damage. Despite the possible differences in the mechanism of action, the efficacy of the endothelin receptor antagonist PD156707 is comparable to the efficacy of the NMDA receptor antagonists (Figure 47).

The marked neuroprotective effects of the endothelin receptor antagonist could be attributed to the increase in cerebral blood flow in the penumbral areas of the focal ischaemic lesion. Recent evidence has suggested the interaction between NMDA

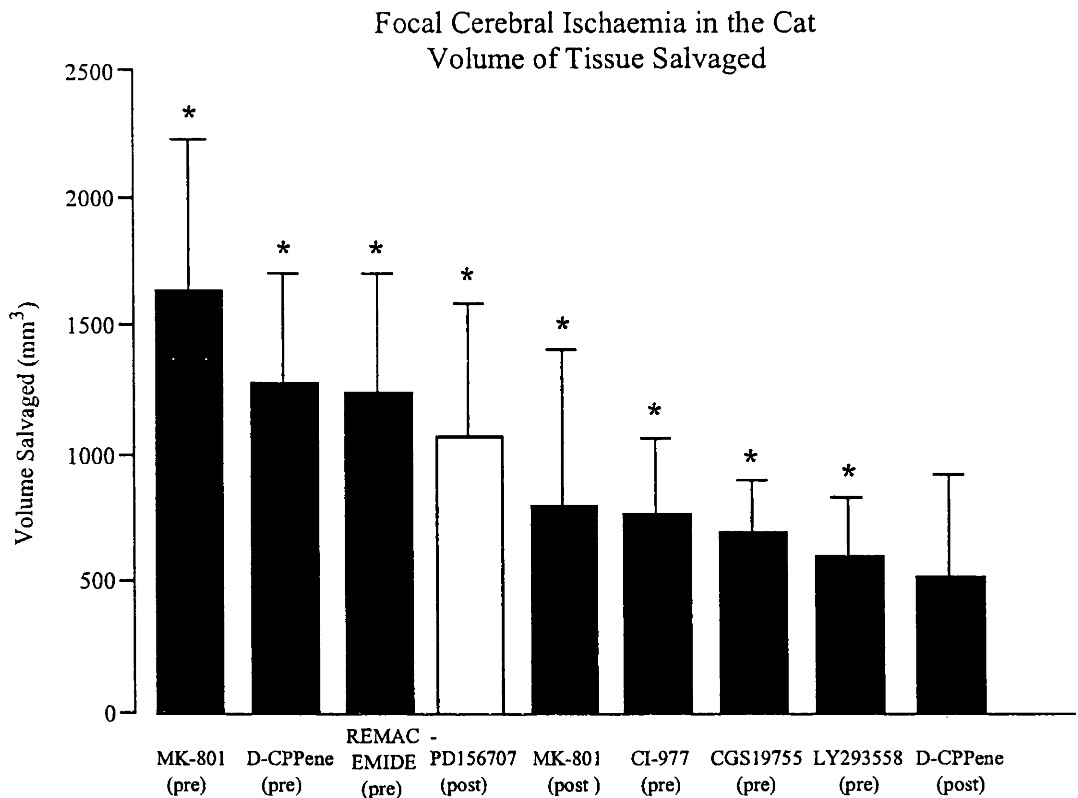


Figure 47 Effect of neuroprotective agents upon the hemispheric volume of ischaemic damage after permanent MCA occlusion in the cat. The data are expressed as the mean ± S.E.M. of volume of tissue salvaged. *P<0.05 compared to vehicle treated animals in each study (one tailed Student's t-test). The drugs were administered as intravenous bolus doses plus continous infusion for 6 hours post-MCAO unless where indicated below. The data are derived from the investigations of:

Ozyurt et al., 1988 MK-801 a non-competitive NMDA antagonist (administered 30 min pre-MCAO)

Bullock et al., 1990 D-CPP-ene a competitive NMDA antagonist (administered 30 min pre-MCAO)

Bannan et al., 1995 Remacemide a non-competitive NMDA antagonist (administered 90 min pre MCAO and discontinuedat MCAO)

Patel et al., in press PD156707 an endothelin ETA receptor antagonist (administered 30 min post-MCAO)

Park et al., 1988 MK-801 a non-competitive NMDA antagonist (administered 2 hours post-MCAO)

Mackay et al., 1994 CI-977 a k-opioid receptor agonist (administered 15 min pre-MCAO)

McCulloch & Bullock, unpublished CGS-19755 a competitive NMDA antagonist (administered 30 min pre-MCAO)

Bullock et al., 1994 LY293558 an AMPA receptor antagonist (administered 30 min pre-MCAO)

Chen et al., 1991 D-CPP-ene a competitive NMDA antagonist (administered 60 min post-MCAO)

receptor activation and endothelin mediated regulation of cardiovascular tone. However, these interactions were observed in the cardiovascular control centres in the brain stem. The failure of endothelin receptor antagonists to alter cerebral blood flow in the normotensive rat suggests that the endothelins have minimal role in normal brain function. The neuroprotective effects of the endothelin receptor antagonists could be attributed to their ability to reduce elevations of intracellular calcium. The endothelin ET_B receptor subtype has been identified on neurones and glia but the significance of these receptors in normal brain function are as yet undetermined.

Global Cerebral Ischaemia

The patterns of neuronal damage associated with global cerebral ischaemia are distinct from the neuronal damage associated with focal cerebral ischaemia (see section 1.2. 3) [Kirino et al., 1982; Kirino et al., 1984]. The main features of global cerebral ischaemia are post-ischaemic hypoperfusion that is observed for the first six hours following ischaemia [Pulsinelli et al., 1982b] and the delayed neuronal damage that is typically observed 24-72 hours following ischaemia. The efficacy of pharmacological agents have been examined in their ability to alleviate the hypoperfusion and reduce the extent of neuronal damage.

The aetiology of the post-ischaemic hypoperfusion is unclear but the L-type calcium channel antagonist nimodipine has been demonstrated to alleviate post-ischaemic hypoperfusion. In a canine model of global cerebral ischaemia, post-ischaemically administered nimodipine alleviated the hypoperfusion despite a reduction in mean arterial blood pressure [Steen et al., 1984]. Investigations in the rat model of global cerebral ischaemia demonstrated a heterogeneous alterations in cerebral blood flow [Kazda et al., 1982; Smith et al., 1984]. Microvascular plugging and constriction of the cerebral arterioles may explain the heterogeneity of the responses [Wade et al., 1975; Grogaard et al., 1989; Moskowitz et al., 1990; Wahl & Schilling, 1993; del Zoppo, 1994; Dirnagl et al., 1994]. No significant link has been observed between the improvement in post-ischaemic hypoperfusion and the improved neurologic function [Smith et al., 1984; Steen et al., 1984].

Delayed neuronal damage associated with global cerebral ischaemia has been reduced by the administration of AMPA receptor antagonists and endothelin receptor antagonist [Gill, 1994; Feuerstein et al., 1994; Ohlstein et al., 1994]. The endothelin receptor antagonist SB209670 and BQ-123 have been demonstrated to reduce the damage of hippocampal CA1 neurones [Feuerstein et al., 1994; Ohlstein et al., 1994]. The delayed administration of AMPA receptor antagonists (between 12-24 hours post global ischaemia) reduces the damage of hippocampal neurones [Li & Buchan, 1993; Xue et al., 1994]. The efficacy of endothelin receptor antagonists when administered immediately post-ischaemia or 12-24 hours following the ischaemic insult has not been examined. NMDA antagonists (MK801) demonstrate reduced efficacy in global cerebral ischaemia when compared to the AMPA receptor antagonists [Buchan et al., 1991; Nellgard et al., 1991; Gill, 1994]. The efficacy of the AMPA receptor antagonists could be the result of their reducing the influx of calcium into the hippocampal neurones. AMPA receptor antagonists depress cerebral glucose use while the NMDA antagonists such as MK-801 elicit hypermetabolic responses in the hippocampal pathway [Kurumaji et al., 1989; Suzdak & Sheardown, 1993; Brown & McCulloch, 1994]. The increases in cerebral metabolism may be associated with the absence of effect of the NMDA receptor antagonists.

The absence of any link between the neurologic function and increases in cerebral blood flow suggests that the two mechanisms play different roles in the development of neuronal damage following global cerebral ischaemia [Steen et al., 1984]. The endothelin receptor antagonist Bosentan failed to alter post-ischaemic hypoperfusion suggesting that the role of the endothelins in the mediation of this phenomenon may be minor. However , the efficacy of the endothelin receptor antagonist in reducing hippocampal neuronal damage is comparable to the effects of the AMPA receptor antagonists [Feuerstein et al., 1994; Ohlstein et al., 1994]. The mechanism of action of the endothelin receptor antagonists may be related to presence of endothelin ET_B receptors on neurones and glia.

4.3 Endothelins: from gene to therapeutic agents

A feature of endothelin research has been the role of molecular biological techniques. The identification of the peptide from porcine endothelial cells was rapidly followed by the identification of the gene codes for the endothelin isopeptides and the endothelin receptors [Arai et al., 1990; Inoue et al., 1989; Sakurai et al., 1990]. The advances in the molecular biology of the endothelins have led to the breeding of transgenic animals with the endothelin genes knocked out. The identification of altered endothelin gene expression has provided circumstantial evidence for the role of the endothelins in the development of a number of disease states (cardiac ischaemia, congestive heart failure, subarachnoid haemorrhage, stroke). The progression from the initial description of the peptide structure to the use of endothelin receptor antagonists as therapeutic agents has been rapid has been rapid (see Table 18).

The role of the endothelins in cardiovascular diseases such as hypertension, congestive heart failure and myocardial ischaemia have been areas of active research. The role of the endothelins in hypertension has been controversial. The use of spontaneously hypertensive strains of rat has demonstrated the increase in plasma endothelin concentrations and the ability of endothelin receptor antagonists to reduce mean arterial blood pressure [Bazil et al., 1992; Hughes et al., 1992; Nishikibe et al., 1993]. In clinical investigations, there is no clear evidence implicating an elevation in plasma endothelin concentrations in the development of hypertension [Battistini et al., 1993].

Plasma concentrations of endothelins increased following experimental cardiac ischaemia (Watanabe et al., 1990; Brunner et al., 1992; Grover et al., 1993; Ray et al., 1992; Brunner, 1995]. In clinical studies similar increases in plasma endothelin-1 levels have reported [Stewart et al., 1991; Lechleitner et al., 1992; Ray et al., 1992]. The efficacy of endothelin receptor antagonists, such as BQ-123 , Bosentan, FR139317, have been examined in experimental cardiac ischaemia and found to reduce the extent of cardiac ischaemic damage [Grover et al., 1993; McMurdo et al., 1994; Wang et al., 1995a; Wang et al., 1995b; Watanabe et al., 1995]. The ability of

Table 18

Progression in endothelin research

1988	Yanagisawa et al. report the isolation of the endothelin peptide.
1989	The isolation of the genes encoding the endothelin isopeptides described.
	Structure of the sarafotoxins (venom from the Israeli burrowing asp) described by Sokolovsky and colleagues.
1990	The mRNA for endothelin ET_A and endothelin ET_B receptors described (Arai et al.; Sakurai et al.).
	Efficacy of endothelin antibodies in experimental myocardial infarction described (Watanabe et al.).
1991	The first description of a peptide endothelin receptor antagonist (BE-18257E) isolated from *Streptomyces misakiensis* (Ihara et al.)
	Preliminary evidence indicating a role for endothelins in SAH and elevated CSF endothelin levels in patients following SAH (Kobayashi et al.; Kraus et al.)
1992	The pharmacology of the endothelin ET_A receptor antagonist BQ-123 and endothelin ET_B agonist BQ-3020 described (Ihara et al.; Ihara et al.).
	Hexapeptide combined endothelin ET_A/ET_B endothelin receptor antagonists PD142893 and PD145065 described (Cody et al.).
	Clinical evidence demonstrating elevated plasma endothelin levels following subarachnoid haemorrhage (Suzuki et al.).
	Elevated plasma endothelin levels in patients following acute stroke (Ziv et al.).
	Elevations in endothelin immunoreactivity in brain tissue following focal cerebral ischaemia (Duverger et al.)
1993	Structure and pharmacology of the first non-peptide combined endothelin ET_A/ET_B endothelin receptor antagonist, Ro46-2005, described (Clozel et al.).
	Bosentan (analogue of Ro46-2005) made available and examined in feline pial arterioles.

Table 18

	Endothelin ET_B receptor mediated vasoconstriction described in some tissues (Shetty et al.; Warner et al.).
	Efficacy of peptide and non-peptide endothelin receptor antagonist in experimental SAH described (Clozel et al.; Clozel & Watanabe; Nirei et al.).
	Efficacy of phosphoramidon in reducing focal ischaemic damage described (Duverger et al.).
1994	Subdivision of endothelin ET_B receptors into ET_{B1} (dilator) and ET_{B2} (constrictor) (Masaki et al.).
	Structure and pharmacology of Bosentan published (Clozel et al.).
	Effects of Bosentan in feline cerebral arterioles described (Patel et al.).
	Pharmacology of non-peptide endothelin receptor antagonists BMS182874 (ET_A antagonist) SB209670 (ET_A/ET_B antagonist) described (Webb et al.; Ohlstein et al.).
	Elevated levels of endothelins in global and focal cerebral ischaemia described.
	BQ-123 and SB209670 reduced hippocampal neuronal damage following global cerebral ischaemia (Feuerstein et al.; Ohlstein et al.).
1995	Structure and pharmacology of non-peptide endothelin receptor antagonists disclosed (PD156707; PD155080) (Doherty et al.).
	PD156707 in reduces neuronal damage and increases cerebral perfusion following focal cerebral ischaemia in the cat.
	Clinical efficacy of bosentan in patients suffering from congestive heart failure (Kiowski et al.).
	Clinical trials of Bosentan in subarachnoid haemorrhage started.

the endothelin receptor antagonist to reduce the myocardial ischaemic damage indicates that these compounds may useful in the clinical context.

Congestive heart failure is usually accompanied by an increase in vascular tone and evidence has indicated that there is an increase in plasma endothelin levels [Cody et al., 1992; Murray et al., 1992; Lerman et al., 1992; Cacoub et al., 1993]. The peptide antagonist FR139317 reduced the systemic blood pressure and peripheral resistance in a canine model of congestive heart failure [Clavell et al., 1995]. The non-peptide antagonist Bosentan has been in clinical trials in patients with congestive heart failure and has been found to reduce the mean arterial blood pressure and vascular resistance in the patients [Kiowski et al., 1995].

The development of the non-peptide endothelin receptor antagonists has facilitated the investigations into the role of the endothelins in cerebrovascular disease. The majority of experimental investigations have focused on the role of endothelin-1 as a mediator of delayed vasospasm following subarachnoid haemorrhage. The basis of these investigations was the detection of elevated plasma and CSF endothelin-1 immunoreactivities and the endothelin receptor antagonists attenuate vasospasm following experimental subarachnoid haemorrhage (see Table 19). The mechanism of action of the endothelins are unclear but increases in endothelin-1 mRNA and alterations in endothelin receptor numbers and subtypes have been observed following subarachnoid haemorrhage [Roux et al., 1995; Shigeno et al., 1995]. The efficacy of the endothelin receptor antagonists in preventing delayed vasospasm associated subarachnoid haemorrhage has been extensively studied (see Table 16). The experimental investigations have demonstrated that the administration of the endothelin receptor antagonists reduces the vasospasm following subarachnoid haemorrhage. As a result of the extensive studies the combined endothelin ET_A/ET_B receptor antagonist Bosentan is currently undergoing clinical trials in subarachnoid haemorrhage.

The endothelin receptor subtypes present in the cerebral circulation in experimental species and the clinical target, man, is an important issue for the development of therapeutic strategies. The available evidence indicates that the

Table 19

Human plasma and CSF endothelin-1 levels measured by radioimmunoassay 7 days following subarachnoid haemorrhage (SAH)

Plasma

Control (pg/ml)	SAH (pg/ml)	Reference
13.2 ± 0.4	18.1 ± 1.3***	Seifert et al., 1995
12.4 ± 0.4	18.7 ± 1.6**	Shirakami et al., 1994
2.6 ± 1.1	~ 5	Ehrenreich et al., 1992
1.1 ± 0.1	7.8 ± 0.9**	Suzuki et al., 1992

CSF

Control (pg/ml)	SAH (pg/ml)	
7.4 ± 1.4	10.1 ± 1.6	Gaetani et al., 1994
9.1 ± 0.2	18.4 ± 2.4**	Shirakami et al., 1994
20.2 ± 1.8	28.3 ± 12.1***	Kraus et al., 1991
	27.9 ± 1.3	Hirata et al., 1990
27.9 ± 2.0	42.1 ± 4.5	Suzuki et al., 1992

Control samples were collected from subjects without any neurological disorders. ** indicates a statistically significant difference from control values ($p<0.01$); *** indicates a statistically significant difference from control values ($p<0.001$)

endothelin ET_A receptor is the predominant constrictor receptor. The receptor subtypes present in human cerebral vessels is less clear but in the peripheral blood vessels the existence of constrictor endothelin ET_A and endothelin ET_B receptors have been demonstrated [Rubanyi & Polokoff et al., 1994; Seo et al., 1994]. The identification of the receptor subtypes in man has important implications for the use of combined endothelin ET_A/ET_B receptor antagonists or antagonists selective for the endothelin ET_A receptor. In experimental subarachnoid haemorrhage there does not appear to be any difference in the efficacy of the combined endothelin ET_A/ET_B receptor antagonists compared to the endothelin ET_A receptor selective receptor antagonists. The endothelin ET_A receptor selective antagonists may be beneficial in attenuating the vasoconstrictor (endothelin ET_A) actions and facilitate endothelin ET_B receptor mediated vasodilatation and a possible increase in cerebral blood flow. In contrast the presence of a mixed population of receptors may require the use of the combined endothelin ET_A/ET_B receptor antagonists. The existence of the blood-brain barrier means that the endothelin receptor antagonists targeted at the cerebral circulation must penetrate the blood brain barrier. The demonstration that the non-peptide endothelin receptor antagonists (Bosentan, PD155080, PD156707), following their intravenous administration, gain access to the cerebrovascular smooth muscle in cerebral cortical arterioles [present thesis] and attenuate vasospasm and ischaemic neuronal damage [Clozel et al., 1993; Roux et al., 1995; Shigeno et al., 1995; Patel et al., in press] demonstrates the utility of these compounds as therapeutic agents.

The endothelin receptor antagonists have been the main focus of drug development but the targeting of endothelin converting enzyme (ECE) may provide another site for the regulation of endothelin synthesis. The efficacy of endothelin receptor antagonists in attenuating cerebral vasospasm and focal ischaemic damage provide a novel therapeutic for the treatment of cerebrovascular diseases. The absence of any significant hypotensive effect in experimental studies indicate that the endothelin receptor antagonists may be of greater therapeutic utility than the agents that induce hypotension on administration, e.g., the L-type calcium channel antagonists (nimodipine). Recent meta-analysis data has indicated that the absence of

any significant effect of nimodipine in stroke patients may have been the result of the delay in the treatment (longer than 12 hours) [Mohr et al., 1994]. The analysis showed that the outcome was improved in patients who were treated within 12 hours of the stroke. The endothelin receptor antagonist PD156707 is effective in experimental studies when administered 30 min post -MCA occlusion and may have a role in the treatment of acute stroke.

The glutamate receptor antagonists have been associated with adverse effects, e.g. hallucinations, in clinical trials and this has precluded their use in the treatment of stroke and head injury [Muir & Lees, 1995]. The effect of endothelin receptor antagonists on cerebral function are unclear but the absence of alterations in cerebral blood flow in experimental investigations suggests that they may not alter cerebral function. The use of neuroprotective agents targeted at the glutamate receptors or inhibitors of glutamate release combined with the endothelin receptor antagonists may provide novel strategies in the treatment of cerebrovascular diseases.

REFERENCES

Abel, P.W., Han, C. (1989) Effects of neuropeptide Y on contraction, relaxation and membrane potential of rabbit cerebral arteries, Journal of Cardiovascular Pharmacology 13, 52-63

Acar, U., Pickard, J. (1978) Effect of angiotensin II on pial arterioles Journal Physiology 284, 859

Adner, M., You, J., Edvinsson, L. (1993) Characterisation of endothelin-A receptors in the cerebral circulation, NeuroReport, 4, 441-445

Alafaci, C., Jansen, I., Arbab, M. A-R. (1989) Enhanced vasoconstrictor effect of endothelin in the cerebral arteries from rats with subarachnoid haemorrhage, Acta Physiologica Scandinavica, 138, 317-319

Alborch, E. (1981) Cholinergic receptors in the cerebral arteries of the goat In, Cerebral microcirculation and metabolism ed. Cervos-Navarro J., Fritschka Raven Press, New York, pp 285-297

Albrightson, C.R., Pullen, M., Wu, H.L., Dytko, G., Hersh, L.B., Nambi, P. (1995) Thrombin-mediated down-regulation of endothelin receptors in mesangial cells coincides with the down-regulation of neutral endopeptidase activity, Molecular Pharmacology, 47, 1156-1163

Allen, J.M., Schon, F., Todd, N., Yeats, J.C., Crockard, H.A., Bloom, S.R. (1984) Presence of neuropeptide Y in human circle of Willis and its possible role in cerebral vasospasm, Lancet, 2 , 550-552

Altura, B.M., Gebrewold, A., Lassoff, S. (1980) Biphasic responsiveness of rat pial arterioles to dopamine: Direct observations on the microcirculation, British Journal of Pharmacology, 69, 543-544

Amara, S.G., Jones, V., Rosenfeld, M.G., Ong, E.S., Evans, R.M. (1982) Alternative RNA processing in calcitonin gene expression generates mRNA encoding different polypeptide products, Nature, 298, 240-244

Ambar, I., Kloog, Y., Schvartz, I., Hazum, E., Sokolovsky, M. (1989) Competitive interaction between endothelin and sarafotoxin, binding and phosphoinositide hydrolysis in rat atria and brain, Biochemical and Biophysical Research Communications, 158, 195-201

Amenta, F., Cavallotti, C., De Rossi, M., Mione, M.C. (1984) Dopamine-sensitive cAMP generating system in rat extracerebral arteries, European Journal of Pharmacology , 97, 1-2, 105-109

Aoki, E., Semba, R., Mikoshiba, K., Kashiwamata, S. (1991) Predominant localisation in glial cells of free L-arginine, immunocytochemical evidence, Brain Research, 547, 190-192

Arai, H., Hori, S., Aramori, I., Ohkubo, H., Nakanishi, S. (1990) Cloning and expression of a cDNA encoding an endothelin receptor, Nature, 348, 730-732.

Archer, S. (1993) Measurement of nitric oxide in biological models, FASEB Journal, 7, 349-360

Armstead, W. M., Mirro, R., Leffler C. W., Busija, D. W. (1989) Influence of endothelin on piglet cerebral microcirculation, American Journal of Physiology, 257, H707-H713

Arunlakshana, O., Schild, H.O. (1959) Some quantitative uses of drug antagonists, British Journal of Pharmacology, 14, 48-58

Astrup, J., Siesjö, B.K. , Symon, L. (1981) Thresholds in cerebral ischaemia - the ischaemic penumbra, Stroke, 12, 723-725.

Astrup, J., Symon, L., Brantson, N.M. , Lassen, N.A. (1977) Cortical evoked potential and extracellular K^+ and H^+ at critical levels of brain ischaemia, Stroke, 8, 51-57.

Auer, L.M., Edvinsson, L, Johansson, B. (1983) Effect of sympathetic nerve stimulation and adrenoceptor blockade on pial arterial and venous calibre and on intracranial pressure in the cat, Acta Physiologica Scandinavica, 119, 213-217

Auer, L.M., Johansson, B.B., Lund, S. (1981) Reaction of pial arteries and veins to sympathetic stimulation in the cat, Stroke, 12, 528-533

Aukland , K., Bower, B.F., Berliner, R.W. (1964) Measurement of local blood flow with hydrogen gas, Circulation Research, 14, 164-187

Baez, S. (1966) Recording of microvascular dimensions with an image splitter television microscope, Journal of Applied Physiology, 21, 299-301.

Balwierczak, J.L (1993) Two subtypes of the endothelin receptor (ET(A) and ET(B)) mediate vasoconstriction in the perfused rat heart, Journal of Cardiovascular Pharmacology, 22 [Suppl. 8], S248-S251

Bamford, J., Sandercock, P. Dennis, M., Burn, J. , Warlow, C. (1990) A prospective study of acute cerebrovascular disease in the community, the Oxfordshire Community Stroke Project, (1981)-86, Journal of Neurology Neurosurgery and Psychiatry, 53, 16-22.

Bannan, P.E., Graham, D.I., Lees, K.R., McCulloch, J. (1994) Neuroprotective effect of remacemide hydrochloride in focal cerebral ischaemia in the cat, Brain Research, 664, 271-275

Barnett, G.H, Bose, B., Little, J.R., Jones, S.C., Friel, H.T. (1986) Effects of nimodipine on acute focal cerebral ischaemia, Stroke, 17, 884-890

Barone, F.C., Globus, M.Y-T., Price, W.J., White, R.F., Storer, B.L., Feuerstein, G.Z., Busto, R., Ohlstein, E.H. (1994) Endothelin levels increase in rat focal and global ischaemia, Journal of Cerebral Blood Flow and Metabolism, 14, 337-342.

Barone, F.C., White, R.F., Elliott, J.D., Feuerstein, G.Z., Ohlstein, E.H. (1995) The endothelin receptor antagonist SB 217242 reduces cerebral focal ischaemic brain injury, Journal of Cardiovascular Pharmacology, 26, S404-S407

Battistini, B., D' Orleans -Juste, P., Sirois, P. (1993), Endothelins , Circulating plasma levels and presence in other biologic fluids, Laboratory Investigation, 68, 600-625

Battistini, B., Warner, T.D., Fournier, A., Vane, J.R. (1994) Characterisation of ET(B) receptors mediating contractions induced by endothelin-1 or IRL 1620 in guinea-pig isolated airways: Effects of BQ-123, FR139317 or PD 145065, British Journal of Pharmacology, 111, 1009-1116

Bax, W., Egbert, B., Saxena, P. (1993) Heterogeneity of endothelin / sarafotoxin receptors mediating contraction of the human isolated saphenous vein, European Journal of Pharmacology, 239, 267-268

Bax, W.A., Saxena, P.R. (1994) The current endothelin receptor classification: Time for reconsideration?, Trends in Pharmacological Sciences, 15, 379-386

Bazil, M.K., Lappe, R.W., Webb, R.L. (1992) Pharmacologic characterisation of an endothelin(A) (ET(A)) receptor antagonist in conscious rats, Journal of Cardiovascular Pharmacology, 20, 940-948

Begley, D.J. (1992) Peptides and the blood-brain barrier In, Physiology and pharmacology of the blood-brain barrier ed. Bradbury MWB Springer Verlag Berlin pp 151-203

Behar, K.L, Rothman, D.L., Hossman, K.A. (1989) NMR spectroscopic investigation of the recovery of energy and acid-base homeostasis in the cat brain after prolonged ischaemia, Journal of Cerebral Blood Flow and Metabolism, 9, 213-245

Benfield, M.C., Korchak, D.M., Roth, B.L., O'Donohue, T.L. (1984) The distribution and chromatographic characterisation of PHI (peptide histidine isoleucine amide) - 27 like peptides in rat and porcine brain, Journal of Neuroscience, 4, 2681-2688

Benveniste, H. (1991) The excitotoxin hypothesis in relation to cerebral ischaemia, Cerebrovascular and Brain Metabolism Reviews, 3, 213-245.

Benveniste, H., Drejer, J., Schousboe, A. , Diemer, N.H. (1984) Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischaemia monitored by intracerebral microdialysis, Journal of Neurochemistry, 43, 1369-1374.

Berrino, L., D'Amico, M., Pizziruso, A., Vitagliano, S., Rossi, F. (1994) Excitatory amino acids modulate the cardiovascular effects of endothelin-1 in the periaqueductal gray area, British Journal of Pharmacology, 111, 173P

Berthiaume, N., Claing, A., Regoli, D., Warner, T.D., D'Orleans-Juste, P. (1995) Characterisation of receptors for kinins and neurokinins in the arterial and venous mesenteric vasculature of the guinea-pig, British Journal of Pharmacology, 115, 1319-1325

Bes, A., Geraud, G., Guell, A., Bes, M.C. (1982) Dopaminergic hypersensitivity in migraine: A diagnostic test?, Nouvelle Presse Medicale, 11, 1475-1478

Bevan, J.A., Buga, G.M., Florence, V.M., Gonsalves, A., Snowden, A. (1982) Distribution of choline acetyltransferase in cerebral and extracerebral cranial arteries of the cat, its relationship to neurogenic atropine-sensitive dilation, Circulation Research, 50, 470-476

Bian, L.G., Zhang, T.X., Zhao, W.G., Shen, J.K., Yang, G.Y. (1994) Increased endothelin-1 in the rabbit model of middle cerebral artery occlusion, Neuroscience Letters, 174, 47-56

Blasberg, R.G., Fenstermacher, J.D., Patlak, C.S. (1983) Transport of a-aminoisobutyric acid across brain capillary and cellular membranes, Journal of Cerebral Blood Flow and Metabolism, 3, 8-32

Bodelsson, G., Stjernquist, M. (1993) Characterisation of endothelin receptors and localization of 125I-endothelin-1 binding sites in human umbilical artery, European Journal of Pharmacology, 249, 299-305

Bogousslavsky, J., Regli, F. (1992) Borderzone infarctions distal to internal carotid artery occlusion, prognostic implications, Annals of Neurology, 20, 346-351

Bonvento, G., Lacombe, P., Seylaz, J. (1989) Effects of electrical stimulation of the dorsal raphe nucleus on local cerebral blood flow in the rat, Journal of Cerebral Blood Flow and Metabolism, 9, 251-255

Bonvento, G., Charbonne, R., Correze, J.L., Borredon, J., Seylaz, J., Lacombe, P. (1994) Is alpha-chloralose plus halothane induction a suitable anaesthetic regimen for cerebrovascular research?, Brain Research, 665, 213-221

Bogousslavsky, J. (1993) Subcortical infarcts In, Current review of cerebrovascular disease eds. Fisher M, Bogousslavsky J. Current Medicine Inc., Philadelphia pp 31-40

Boysen, G. (1993) Primary intracerebral haemorrhage In, Current review of cerebrovascular disease eds. Fisher M, Bogousslavsky J. Current Medicine Inc., Philadelphia pp 78-88

Bradbury, M. (1979) The concept of a blood-brain barrier John Wiley , sons

Brandt, L, Ljunggren, B., Persson, K-E., Edvinsson, L., MacKenzie, E., Tamura, A., Teasdale, G. (1983) Effect of topical application of a calcium antagonist (nifedipine) on feline cortical pial microvasculature under normal conditions and in focal ischaemia, Journal of Cerebral Blood Flow and Metabolism, 3, 44-50.

Branston, N.M., Strong, A.J. , Symon, L. (1977) Extracellular potassium activity, evoked potential and tissue blood flow, Journal of Neurological Sciences, 32, 305-321.

Branston, N.M., Symon, L., Crockard, H.A., Pasztor, E. (1974) Relationship between the cortical evoked potential and local blood flow following acute middle cerebral artery occlusion in the baboon, Experimental Neurology, 45, 195-208.

Bredt, D.S., Hwang, P.M., Snyder, S.H. (1990) Localisation of nitric oxide synthase indicating a neural role for nitric oxide, Nature, 347, 768-770

Brightman, M.W. (1992) Ultrastructure of brain endothelium In, Physiology and pharmacology of the blood-brain barrier ed. Bradbury MWB Springer Verlag Berlin pp 1-22

Brown, M.R., Vaughan, J., Jimenez, L.L., Vale, W., Baird, A. (1991) Transforming growth factor-B: role in mediating serum induced endothelin production by vascular endothelial cells, Endocrinology, 129, 2355-2360

Brown, R.D., Wiebers, D.O. (1993) Subarachnoid haemorrhage In, Current review of cerebrovascular disease eds. Fisher, M,, Bogousslavsky, J. Current Medicine Inc., Philadelphia, pp 89-99

Browne, S.E. , McCulloch, J. (1994) AMPA receptor antagonists in local cerebral glucose utilisation in the rat, Brain Research, 641, 10-20.

Brunner, F. (1995) Cardiac tissue endothelin-1 levels under basal, stimulated, and ischaemic conditions, Journal of Cardiovascular Pharmacology, 26 (Suppl. 3), S44-S46

Brunner, F., du Toit, E.F., Opie, L.H. (1992) Endothelin release during ischaemia and reperfusion of isolated perfused rat hearts, Journal of Molecular and Cellular Cardiology, 24, 1291-1305

Buchan, A.M., Xue, D., Huang, Z.-G., Smith, K.H. and Lesiuk, H. (1991) Delayed AMPA receptor blockade reduces cerebral infarction induced by focal ischaemia, NeuroReport, 2, 473-476.

Bullock, R. , Teasdale, G.M. (1990) Surgical management of traumatic intracranial haematomas, in, Handbook of Clinical Neurology. Head Injury, Vol. 24, Ed. R. Braakman, Elsevier, Amsterdam, pp. 95-118.

Bullock, R., Butcher, S.P, Chen, M.-H., Kendal, L., McCulloch, J. (1991) Correlation of the extracellular glutamate concentration with extent of blood flow reduction after subdural haematoma in the rat, Journal of Neurosurgery, 74, 794-802

Bullock, R., Fujisawa, H. (1992) The role of glutamate antagonists for the treatment of CNS injury, Journal of Neurotrauma, 9, S443-S462

Bullock, R., Graham, D.I., Chen, M.-H., Lowe, D. , McCulloch, J. (1990) Focal cerebral ischaemia in the cat, Pretreatment with a competitive NMDA receptor antagonist, D-CPPene, Journal of Cerebral Blood Flow and Metabolism, 10, 668-674.

Bullock, R., Graham, D.I., Swanson, S. , McCulloch, J. (1994) The neuroprotective effect of the AMPA receptor antagonist LY-293558 in focal cerebral ischaemia in the cat, Journal of Cerebral Blood Flow and Metabolism, 14, 466-471.

Busto, R., Dietrich, W.D., Globus, M. Y-T., Valdés, I., Scheinberg, P. , Ginsberg, M.D. (1987) Small differences in intraischaemic brain temperature critically determine the extent of ischaemic neuronal injury, Journal of Cerebral Blood Flow and Metabolism, 7, 729-738.

Butcher, S.P., Bullock, R., Graham, D.I. , McCulloch, J. (1990) Correlation between amino acid release and neuropathologic outcome in rat brain following middle cerebral artery occlusion, Stroke, 21, 1727-1733.

Cacoub, P., Dorent, R., Nataf, P., Carayon, A., Maistre, G., Piette, J.C., Godeau, P., Cabrol, C., Gandjbakhch, I. (1993) Plasma endothelin and pulmonary pressures in patients with congestive heart failure, American Heart Journal, 126, 1484-1488

Cao, L.Q., Banks, R.O. (1990) Cardiovascular and renal actions of endothelin: effects of calcium channel blockers, American Journal of Physiology, 258, 254-258

Cardell, L.O., Uddman, R., Edvinsson, L. (1990) Analysis of endothelin-1 induced contractions of guinea pig trachea, pulmonary vein and different types of pulmonary arteries, Acta Physiologica Scandinavica, 139, 103-111

Cardell, L.O., Udmann, R., Edvinsson, L. (1993) A novel ETA receptor antagonist FR139317 inhibits endothelin induced contractions of Guinea pig pulmonary arteries, but not trachea, British Journal of Pharmacology, 108, 448-455

Casscells, W., Bazoberry, F., Speir, E., Thompson, N., Flanders, K., Kondaiah, P., Ferrans, V.J., Epstein, S.E., Sporn, M. (1990) Transforming growth factor-beta1 in normal heart and in myocardial infarction, Annals of the New York Academy of Sciences, 593, 148-160

Cavero, P.G., Miller, W.L., Heublein, D.M., Margulies, K.B., Burnett, J.C. (1990) Endothelin in experimental congestive heart failure in the anaesthetised dog, American Journal of Physiology, 259, F312-F317

Chang, H., Wu, G.J., Wang, S.M., Hung, C.R. (1993) Plasma endothelin levels and surgically correctable pulmonary hypertension, Annals of Thoracic Surgery, 55, 450-458

Chang, J.Y., Hardebo, J.E., Owman, C. (1988) Differential vasomotor action of noradrenaline, serotonin, and histamine in isolated basilar artery from rat and guinea-pig, Acta Physiologica Scandinavica, 132, 91-102

Changaris, D.G., Severs, W.B., Keil, L.C. (1978) Localisation of angiotensin in rat brain, Journal of Histochemistry and Cytochemistry, 26, 593-607

Checkley, D., Pleeth, R., Breen, S., Waterton, J., Wilson, C. (1995) Effect of BQ123 on rat cerebral infarct size measured by diffusion- and T2- weighted MRI (abstract no. P174) 4th International conference on Endothelin, 23-26 April; London U.K.

Chen, M., Bullock, R., Graham, D.I., Frey, P., Lowe, D., McCulloch, J. (1991a) Evaluation of a competitive NMDA antagonist (D-CPPene) in feline focal cerebral ischaemia. Annals of Neurology, 30, 62-70

Chen, M.-H., Bullock, R., Graham, D.I., Miller, J.D., McCulloch J. (1991b) Ischaemic neuronal damage after acute subdural haematoma in the rat: effects of pre-treatment with a glutamate antagonist, Journal of Neurosurgery, 74, 944-950

Choi, D.W. (1988) Glutamate neurotoxicity and diseases of the nervous system, Neuron, 1, 623-634.

Choi, D.W. (1990) Methods for antagonising glutamate neurotoxicity, Cerebrovascular Brain Metabolism Reviews, 2, 105-147.

Chopp, M., Welch, K.M.A., Tidwell, C.D. , Helpern, J.A. (1988) Global cerebral ischaemia and intracellular pH during hyperglycaemia and hypoglycaemia in cats, Stroke, 19, 1383-1387.

Chou, S.Y., Dahlan, A., Porrush, J.G. (1990) Renal actions of endothelin: interaction with prostacyclin, American Journal of Physiology, 259, F645-F652

Clavell, A., Stingo, A., Margulies, K., Lerman, A., Underwood, D., Burnett, J.C. (1993) Physiological significance of endothelin: its role in congestive heart failure, Circulation, 87 (Suppl. 6), III 12- III 19

Clavell, A.L., Stingo, A.J., Margulies, K.B., Brandt, R.R., Burnett, J.C. (1995) Role of endothelin receptor subtypes in the in vivo regulation of renal function, American Journal of Physiology, 268, F455-F460

Clozel, M., Breu, V., Burri, K., Cassal, J-M., Fischli, W., Gray, G.A., Hirth, G., Loffler, B-M., Muller, M., Neidhart, W., Ramuz, H. (1993) Pathophysiological role of endothelin revealed by the first orally active endothelin receptor antagonist, Nature, 365, 759-763.

Clozel, M., Breu, V., Gray, G.A., Kalina, B., Loffler, B-M., Burri, K., Cassal, J-M., Hirth, G., Muller, M., Neidhart, W., Ramuz, H. (1994) Pharmacological characterisation of bosentan, a new potent orally active non-peptide endothelin receptor antagonist, Journal of Pharmacology and Experimental Therapeutics, 270, 228-235

Clozel, M., Clozel, J -P. (1989) Effects of endothelin on regional cerebral blood flow in squirrel monkeys, Journal of Pharmacology and Experimental Therapeutics, 250, 1125-1131

Clozel, M., Gray, G.A. (1995) Are there different ET(B) receptors mediating constriction and relaxation?, Journal of Cardiovascular Pharmacology, 26 [Suppl. 3], S262-S264

Clozel, M., Gray, G.A., Breu, V., Loffler, B-M., Osterwalder, R. (1992) The endothelin ETB receptor mediates vasodilatation and vasoconstriction in vivo, Biochemical and Biophysical Research Communications, 186, 867-873.

Clozel, M., Loffler, B.M., Ghor, H. (1991) Relative preservation of the responsiveness to endothelin-1 during reperfusion following renal ischaemia, Journal of Cardiovascular Pharmacology, 17 (Suppl. 7): S313-S315

Clozel, M., Watanabe, H. (1993) BQ123, a peptidic endothelin ETA receptor antagonist prevents the early vasospasm following subarachnoid hemorrhage after intracisternal but not intravenous injection, Life Sciences, 52, 825,834.

Cody, W.L., Doherty, A.M., He, J.X., DePue, P.L., Waite, L.A., Topliss, J.G., Haleen, S.J., LaDouceur, D., Flynn, M.A., Hill, K.E., Reynolds, E.E. (1993) The rational design of a highly potent combined ETA and ETB receptor antagonist (PD145065) and related analogues, Medicinal Chemistry Research, 3, 154-162

Collingridge, G.L. , Bliss, T.V.P. (1987) NMDA receptors - their role in long-term potentiation, Trends in Neuroscience, 10, 288-293.

Cosentino, F., McMahon, E.G., Carter, J.S. (1993) Effect of endothelin A-receptor antagonist BQ123 and phosphoramidon on cerebral vasospasm, Journal of Cardiovascular Pharmacology, 22 (Suppl. 8), S332-S335

Coyle, P., Jokelainen, P.T. (1982) Dorsal cerebral arterial collaterals of the rat. Anatomical Record, 203, 397-404.

Cristol, J.P., Warner, T.D., Thiermermann, C., Vane, J.R. (1993) Mediation via different receptors of the vasoconstrictor effects of endothelins and sarafotoxins in the systemic circulation and renal vasculature of the anaesthetised rat, British Journal of Pharmacology, 108, 776-779

D'Orleans-Juste, P., Telemaque, S., Claing, A., Ihara, M., Yano, M. (1992) Human big-endothelin-1 and endothelin-1 release prostacyclin via the activation of ET-1 receptors in the rat perfused lung, British Journal of Pharmacology, 105, 773-775

Dagassan, P.H, Clozel, M., Breu, V., Clozel, J.P. (1995) Role of endothelin in spontaneous phasic contraction of human coronary arteries, Journal of Cardiovascular Pharmacology, 26 [Suppl. 3], S200-S203

Daly, R.N., Hieble, J.P. (1987) Neuropeptide Y modulates adrenergic neurotransmission by an endothelium dependent mechanism, European Journal of Pharmacology, 138, 445-446

Date, H., Hossmann, K.A. (1984) Effect of vasodilating drugs on intracortical and extracortical vascular resistance following middle cerebral artery occlusion in cats, Annals of Neurology, 16, 330 -336

Dauphin, F., MacKenzie, E.T. (1995) Cholinergic and vasoactive intestinal polypeptidergic innervation of the cerebral arteries, Pharmacy and Therapeutics, 67, 385-417

Dauphin, F., Ting, V., Payette P., Dennis, M., Hamel, E. (1991) Vasocontractile M1 muscarinic receptors in cat cerebral arteries, pharmacological identification and detection of mRNA, European Journal of Pharmacology (Molecular Pharmacology Sect), 207, 319-327

Davenport, A.P., Kuc, R.E., Maguire, J.J., Harland, S.P. (1995) ET(A) receptors predominate in the human vasculature and mediate constriction, Journal of Cardiovascular Pharmacology, 26 [Suppl. 3], S265-S267

Davenport, A.P., Maguire, J.J. (1994) Is endothelin-induced vasoconstriction mediated only by ET(A) receptors in humans?, Trends in Pharmacological Sciences, 15, 9-11

Davenport, A.P., Nunez, D.J., Brown, M.J. (1989) Binding sites for 125I labelled endothelin-1 in the kidneys: differential distribution in rat, pig, man demonstrated by using autoradiography, Clinical Science, 77, 129-131

Davenport, A.P., Nunez, D.J., Brown, M.J. (1990) Localisation of binding sites for iodinated endothelin and sarafotoxin peptides in mammalian tissues using quantitative receptor autoradiography, European Journal of Pharmacology, 183, 2153

De Ley, G., Weyne, J., Demeester, G., Leusen, I. (1984) Response of local blood flow in the caudate nucleus of the cat to intraventricular administration of carbachol, Stroke 15, 481-486

De Lombaert, S., Ghai, R.D., Jeng, A.Y., Trapani, A.J., Webb, R.L. (1995) Pharmacological profile of a non-peptidic dual inhibitor of neutral endopeptidase 24.11 and endothelin-converting enzyme, Biochemical and Biophysical Research Communications, 204, 407-412

De Nucci, G., Thomas, R., D'Orleans-Juste, P., Antunes, E., Walder, C., Warner, T.D., Vane, J.R. (1988) Pressor effects of circulating endothelin are limited by its removal in the pulmonary circulation and by the release of prostacyclin and endothelium derived relaxing factor, Proceedings of the National Academy of Sciences U.S.A., 85, 9797-9800

De Oliveira, A.M., Viswanathan, M., Capsoni, S. (1995) Characterisation of endothelin A receptors in cerebral and peripheral arteries of the rat, Peptides, 16, 139-194

de Courten-Meyers, G., Myers, R.E. , Scholfield, L. (1988) Hyperglycaemia enlarges infarct size in cerebrovascular occlusion in cats, Stroke, 19, 623-630.

del Zoppo, G.J. (1994) Microvascular changes during cerebral ischaemia and reperfusion, Cerebrovascular and Brain Metabolism Reviews, 6, 47-96.

Derouesne, C., Cambon, H., Yelink, A., Duydkaerts, C., Hauw, J.J. (1993) Infarcts in the middle cerebral artery territory, Acta Neurologica Scandinavica, 87, 361-366.

Deschepper, C.F., Houweling, A.D., Picard, S. (1995) The membranes of cultured rat brain astrocytes contain endothelin-converting enzyme activity, European Journal of Pharmacology, 275, 61-66

Dieguez, G., Garcia, J.L, Fernandez, N., Garcia-Villalon, A.L., Monge, L., Gomez, B. (1992) Cerebrovascular and coronary effect of endothelin-1 in the goat, American Journal of Physiology, 263, R834-R839

Dirnagl U, Niwa N, Sixt G, Villringer A (1994) Cortical hypoperfusion after global forebrain ischaemia in rats is not caused by microvascular plugging, Stroke, 25, 1028-1038.

Doherty, A.M. (1992) Endothelin, a new challenge, Journal of Medicinal Chemistry, 35, 1493-1508.

Doherty, A.M., Patt, W.C., Edmunds, J.J., Berryman, K.A., Reisdorph, B.R., Plummer, M.S., Shahripour, A., Lee, C., Cheng, X-M., Walker, D.M., Haleen, S.J., Keiser, J.A., Flynn, M.A., Welch, K.M., Hallak, H., Taylor, D.G., Reynolds, E.E. (1995) Discovery of a novel series of orally active non peptide endothelin-A (ETA) receptor selective antagonists, Journal of Medicinal Chemistry, 38, 1259-1263

Dohi, Y., Luscher, T.F. (1991) Endothelin in hypertensive resistance arteries: intraluminal and extraluminal dysfunction, Hypertension, 10, 543-549

Douglas, S.A., Beck, G.R., Elliott, J.D., Ohlstein, E.H. (1995) Pharmacologic evidence for the presence of three functional endothelin receptor subtypes in rabbit saphenous vein, Journal of Cardiovascular Pharmacology, 26 (Suppl. 3), S163-S168

Doyle, T.F., Martins, A.N., Kobrine, A.I. (1975) Estimating total cerebral blood flow from the initial slope of hydrogen washout curves, Stroke, 6, 149-152

Duckles, S.P. (1982) Modulation of endogenous noradrenaline release by prejunctional α-adrenoceptors, comparison of a cerebral and peripheral artery, Journal of Autonomic Pharmacology, 2, 71-77

Duckles, S.P., Kennedy, C.D. (1982) Cerebral blood vessels, effects of exogenous acetylcholine and field stimulation on norepinephrine release, Journal of Pharmacology and Experimental Therapeutic, 222, 562-565

Durieu-Trautmann, O., Federici, C., Creminon, C., Foignant-Chaverot, N., Roux, F., Claire, M., Strosberg, A.D., Couraud, P.O. (1993) Nitric oxide and endothelin secretion by brain microvessel endothelial cells, Regulation by cyclic nucleotides, Journal of Cellular Physiology, 155, 104-111

Duverger, D., Dennis, T., Edvinsson, L., MacKenzie, E.T., Oblin, A., Scatton, B., Zivkovic, (1987) Concentrations of putative neurovascular transmitters in major cerebral arteries and small pial vessels of various species, Journal of Cerebral Blood Flow and Metabolism,

Duverger, D., Viossat, I., Chapelat, M., Chabrier, P-E., Pirotsky, E., Braquet, P. (1992) Focal ischaemia in the rat, measurement of brain tissue endothelin [in French], Circ Metab Cerveau, 9, 85-93.

Duverger, D., Viossat, I., Chapelat, M., Chabrier, P-E., Pirotzky, E., Braquet, P. (1993) Effects of phosphoramidon on immunoreactive ET-1 contents in brain and volume of infarctus in MCA-occluded rat, Journal of Cerebral Blood Flow and Metabolism, 13 (Suppl. 1), S194

Dux, E., Joo, F. (1982) Effects of histamine on brain capillaries. Fine structural and immunohistochemical studies after intracarotid infusion, Experimental Brain Research, 47, 252-258

Edvinsson, L., Jansen, I. (1987) Characterisation of tachykinin receptors in isolated cerebral arteries of guinea pig, British Journal of Pharmacology, 90, 553-559

Edvinsson, L. (1982) Sympathetic control of cerebral circulation, Trends in Neurosciences, 5, 425-429

Edvinsson, L. (1985) Characterisation of the contractile effects of neuropeptide Y in feline cerebral arteries, Acta Physiologica Scandinavica, 125, 33-41

Edvinsson, L., Copeland, J., Emson, P.C., McCulloch, J., Uddman, R. (1987) Nerve fibres containing neuropeptide Y in the cerebrovascular bed, immunocytochemistry, radioimmunoassay and vasomotor effects, Journal of Cerebral Blood Flow and Metabolism, 7, 45-57

Edvinsson, L., Emson, P., McCulloch, J., Tatemoto, K., Uddman, R. (1983) Neuropeptide Y, cerebrovascular innervation and vasomotor effects in the cat, Neuroscience Letters, 43, 79-84

Edvinsson, L., Emson, P.C., McCulloch, J., Tatemoto, K., Uddman, R. (1984) Neuropeptide Y, immunocytochemical localisation to and effect upon feline pial arteries and veins in vitro and in situ, Acta Physiologica Scandinavica, 122, 155-163

Edvinsson, L., Falck, B., Owman, C. (1977) Possibilities for a cholinergic action on smooth musculature and on sympathetic axons in brain vessels mediated by muscarinic and nicotinic receptors, Journal of Pharmacology and Experimental Therapeutics, 200, 117-126

Edvinsson, L., Fredholm, B.B., Hamel, E., Jansen, I., Verrecchia, C. (1985a) Perivascular peptides relax cerebral arteries concomitant with stimulation of cyclic adenosine monophosphate accumulation or release of an endothelium derived relaxing factor in the cat, Neuroscience Letters 58, 213-217

Edvinsson, L., Hara, H., Uddman, R. (1989) Retrograde tracing of nerve fibres to the rat middle cerebral artery with true blue; co-localisation with different peptides, Journal of Cerebral Blood Flow and Metabolism, 9, 212-218

Edvinsson, L., Hardebo, J.E., Owman, C. (1979) Effect of angiotensin II on cerebral blood vessels, Acta Physiologica Scandinavica, 105, 381-383

Edvinsson, L., MacKenzie, E.T. (1977) Amine mechanisms in the cerebral circulation, Pharmacology Reviews, 28, 275-348.

Edvinsson, L., MacKenzie, E.T., McCulloch, J. (1993) in Cerebral Blood Flow and Metabolism Raven Press New York

Edvinsson, L., MacKenzie, E.T., McCulloch, J. (1993) Neuropeptides In, Cerebral blood flow and metabolism Raven press New York, pp 231-237

Edvinsson, L., McCulloch, J. (1985) Distribution and vasomotor effects of peptide HI (PHI)on feline cerebral blood vessels in vitro and in situ, Regulatory Peptides, 10, 345-356

Edvinsson, L., McCulloch, J., Rosell, S., Uddman, R. (1982) Antagonism by (D-Pro^2, D-Pro^{7,9}) substance P of the cerebrovascular dilation induced by substance P, Acta Physiologica Scandinavica, 116, 411-416

Edvinsson, L., McCulloch, J., Sharkey, J. (1985b) Vasomotor responses of cerebral arteries in situ to putative dopamine agonists, British Journal Pharmacology, 85, 403-410

Edvinsson, L., McCulloch, J., Uddman, R. (1981) Substance P, immunohistochemical localisation and effect upon feline pial arteries in vitro and in situ, Journal of Physiology, 318, 251-258

Edvinsson, L., Owman, C. (1974) Pharmacological characterisation of adrenergic alpha and beta receptors mediating the vasomotor responses of cerebral arteries in vitro, Circulation Research 35, 835-840

Edwards, R., Trizna, W. (1990) Response of isolated intracerebral arterioles to endothelins, Pharmacology, 41, 149-152

Egashima, K., Pipers, F.S., Rush, J.E., Morgan, J.P. (1990) Effects of calcium channel blockers on coronary vasoconstriction induced by endothelin-1 in closed chest pigs, Journal of the American College of Cardiologists, 16, 1296-1303

Eglezos, A., Cucchi, P., Patacchini, R., Quartara, L., Maggi, C.A., Mizrahi, J. (1993) Differential effects of BQ-123 against endothelin-1 and endothelin-3 on the rat vas deferens: Evidence for an atypical endothelin receptor, British Journal of Pharmacology, 109, 736-738

Ehrenreich, H., Kehrl, J., Anderson, R., Rieckmann, P., Vitkovic, L., Coligan, J., Fauci, A. (1991) A vasoactive peptide, endothelin-3 is produced by and specifically binds to primary astrocytes, Brain Research, 538, 54-58.

Ehrenreich, H., Lange, M., Near, K.A., Anneser, F., Schoeller, L.A.C., Schmid, R., Winkler, P.A., Kehrl, J.H., Schmiedek, P., Goebel, F.D. (1992) Long-term monitoring of immunoreactive endothelin-1 and endothelin-3 in ventricular cerebrospinal-fluid, plasma, and 24-h urine of patients with subarachnoid hemorrhage, Research in Experimental Medicine, 192, 257-268

Ekelund, U., (1994) In vivo effects of endothelin-2, endothelin-3 and ET(A) receptor blockade on arterial, venous and capillary functions in the cat skeletal muscle, Acta Physiologica. Scandinavica, 150, 147-153

Ekelund, U., Adner, M., Edvinsson, L., Mellander, S. (1995) Effects of the combined ET(A) and ET(B) receptor antagonist PD145065 on arteries, arterioles and veins in the cat hindlimb, Journal of Cardiovascular Pharmacology, 26 (Suppl. 3), S211-S213

Ekelund, U., Albert, U. , Edvinsson, L., Mellander, S. (1993) In vivo effects of endothelin-1 and ET(A) receptor blockade on arterial, venous and capillary functions in skeletal muscle, Acta Physiologica Scandinavica, 148, 273-278

Elton, T.S., Oparil, S., Taylor, G.R., Hicks, P.H., Yang, R-H., Jin, H., Chen, Y.F. (1992) Normabaric hypoxia stimulates endothelin-1 gene expression in the rat, American Journal of Physiology, 263, R1260-R1264

Emori, T., Hirata, Y., Kanno, K., Ohta, K., Eguchi, S , Imai, T., Shichiri, M., Marumo, F. (1991a) Endothelin-3 stimulates production of endothelium-derived nitric oxide via phosphoinositide breakdown, Biochemical and Biophysical Research Communications, 174, 228-235

Emori, T., Hirata, Y., Marumo, F. (1991b) Endothelin-3 stimulates prostacyclin production in cultured bovine endothelial cells, Journal of Cardiovascular Pharmacology, 17 (Suppl. 7), S140-S142

Encabo, A., Ferrer, M., Marin, J., Villamor, J., Balfagon, G. (1992) Vasoconstrictive responses elicited by endothelin in bovine cerebral arteries, General Pharmacology, 23, 263-267

Endo, T., Uchida, Y., Matsumoto, H., Suzuki, N., Nomura, A., Hirata, F., Hasegawa, S. (1992) Regulation of endothelin-1 synthesis in cultured guinea pig airway epithelial cells by various cytokines, Biochemical and Biophysical Research Communications, 186, 1594-1599

Ermisch, A., Brust, P., Kretzschmar, R., Ruhle, H-J. (1993) Peptides and blood brain barrier transport, Physiology Reviews, 73, 489-527

Estrada, V., Tellez, M.J., Moya, J., Fernandez-Durango, R., Egido, J., Cruz, A.F. (1994) High plasma levels of endothelin-1 and atrial natriuretic peptide in patients with acute ischaemic stroke, American Journal of Hypertension, 7, 1085-1089

Fabricius, M., Lauritzen, M. (1994) Examination of the role of nitric oxide for the hypercapnic rise of cerebral blood flow in rats, American Journal of Physiology, 266, H1457-H1464

Fabricius, M., Lauritzen, M. (1995) Laser doppler flowmetry overestimates increases of rat cerebral blood flow as compared to the 14C-iodoantipyrine method, Journal of Cerebral Blood Flow and Metabolism, 15 (Suppl. 1), S634

Fallgren, B., Arlock, P., Jansen, I., Edvinsson, L. (1990) Neuropeptide Y in cerebrovascular function, comparison of membrane potential changes and vasomotor responses evoked by NPY and other vasoconstrictors in the guinea pig basilar artery, Neuroscience Letters, 114, 117-122

Fallgren, B., Ekblad, E., Edvinsson, L. (1989) Co-existence of neuropeptide and differential inhibition of vasodilator responses by neuropeptide Y in guinea pig uterine arteries, Neuroscience Letters, 100, 71-76

Faraci, F.M. (1989) Effects of endothelin and vasopressin on cerebral blood vessels, American Journal of Physiology, 257, H799-H803

Faraci, F.M., Breese, K.R. (1993) Nitric oxide mediates vasodilation in response to activation of N-Methyl D-Aspartate receptors in brain, Circulation Research, 72, 476-480

Faraci, F.M., Brian, J.E. (1994) Nitric oxide and the cerebral circulation, Stroke, 25, 692-703

Faro, R., Grassi-Kassisse, D.M., Donato, J.L., Boin, I., Opgenorth, T.J., Withrington, P.G., Zatz, R., Antunes, E., De Nucci, G. (1995) Role of endothelin ET(A) and ET(B) receptors in the arterial vasculature of the isolated canine liver, Journal of Cardiovascular Pharmacology, 26 [Suppl. 3], S204-S207

Federici, C., Camoin, L., Creminon, C., Chaverot, N., Strosberg, A.D., Couraud, P.O. (1995) Cultured astrocytes release a factor that decreases endothelin-1 secretion by brain microvessel endothelial cells, Journal of Neurochemistry, 64, 1008-1015

Feger, G.I., Schilling, L., Ehrenreich, H., Wahl, M. (1994) Endothelin-induced contraction and relaxation of isolated rat basilar artery, Effect of BQ-123, Journal of Cerebral Blood Flow and Metabolism, 14, 845-852

Feischi, C., Bozzao, A., Agnoli, A. (1969) The hydrogen clearance method of measuring local blood flow in subcortical structures of the brain, including a comparative study with 14C-antipyrine method, Experimental Brain Research, 7, 111-119

Ferguson, A.V. (1995) Central nervous system actions of endothelins in cardiovascular control In: Endothelin: role in health and disease ed. Gulati, A. Harwood Academic publishers Germany, pp 143-153

Ferguson, A.V., Smith, P. (1990) Cardiovascular responses induced by endothelin microinjection into area postrema, Regulatory Peptides, 27, 75-85

Ferrer, M., Encabo, A., Marin, J., Peiro, C., Redondo, J., Desagarra, M.R., Balfagon, G. (1992) Comparison of the vasoconstrictor responses induced by endothelin and phorbol 12,13-dibutyrate in bovine cerebral-arteries, Brain Research, 599, 186-196

Feuerstein, G., Gu, J.L., Ohlstein, E.H., Barone, F.C., Yue, T.L. (1994) Peptidic endothelin-1 receptor antagonist, BQ-123, and neuroprotection, Peptides, 15, 467-469

Firth, J.D., Roberts, A.F., Raine, A.E. (1990) Effect of endothelin on the function of the isolated perfused working rat heart, Clinical Science, 79, 221-226

Fisher, M., Grotta,J. (1993) New uses for calcium channel blockers, Therapeutic implications, Drugs, 46, 961-975

Fitch, W., Edvinsson, L., Watson, R., Watt, P., McCulloch, J. (1983) Influences of pentobarbital and a-chloralose on cerebrovascular responses to vasoactive agents, Journal of Cerebral Blood Flow and Metabolism, 3 (Suppl. 1), S520-S521

Foley, P.L., Caner, H.H., Kassell, N.F., Lee, K-S. (1994) Reversal of subarachnoid hemorrhage-induced vasoconstriction with an endothelin receptor antagonist, Neurosurgery, 34, 108-113

Foster, A.C. , Fagg, G.E. (1987) Taking apart NMDA receptors, Nature, 329, 395-396.

Fozard, J.R., Part, M.L. (1992) The role of nitric oxide in the regional vasodilator effects of endothelin-1 in the rat, British Journal of Pharmacology, 105, 744-750

Frelin, C., Ladoux, A., Marsault, R., Vigne, P. (1992) Function of vasoactive factors in the cerebral microcirculation, Journal of Cardiovascular Pharmacology, 20 (Suppl. 12), S94-S96

Frerichs, K.U., Siren, A.L., Feuerstein, G.Z., Hallenbeck, J.M. (1992) The onset of post ischaemic hypoperfusion in rats is precipitous and may be controlled by local neurones, Stroke, 23, 399-406.

Fujimori, A., Yanagisawa, M., Saito, A., Goto, K., Masaki T., Mima, T., Takakura, K. (1990) Endothelin in plasma and cerebrospinal fluid of patients with subarachnoid haemorrhage, Lancet, 336, 633.

Fukami T, Hayama T, Amano Y, Nakamura Y, Arai Y, Matsuyama K, Yano M, Ishikawa K (1994) Aminophosphonate endothelin converting enzyme inhibitors, potency enhancing and selectivity improving modifications of phosphoramidon, Bioorganic and Medicinal Chemistry Letters, 4, 1257-1262

Fukuda, S., Taga, K., Shimoji, K. (1991) High sensitivity of porcine cerebral arteries to endothelin, Experientia, 47, 475-477

Fukunaga, M, Fujiwara, Y., Ochi, S., Yokoyama, K., Fujibayashi, M., Orita, Y., Fukuhara, Y., Ueda, N., Kamada, T. (1991) Stimulatory effect of thrombin on endothelin-1 production in isolated glomeruli and cultured mesangial cells of rats, Journal of Cardiovascular Pharmacology, 17 (Suppl. 7), S411-S413

Fukuroda, T., Nishikibe, M., Ihara, M., Yano, M., Ishikawa, K., Ikemoto, F. (1992) Effects of a novel endothelin antagonist, BQ-153, on endothelin-induced vasoconstriction in porcine blood vessels, Japanese Journal of Pharmacology, 58 [Suppl. 2], 278P

Fukuroda, T., Noguchi, K., Tsuchida, S., Nishikibe, M., Ikemoto, F., Okada, K., Yano, M. (1990) Inhibition of biological actions of big endothelin-1 by phosphoramidon, Biochemical Biophysical Research Communication, 172, 390-395

Fukuroda, T., Ozaki, S., Ihara, M., Ishikawa, K., Yano, M., Nishikibe, M. (1994) Synergistic inhibition by BQ-123 and BQ-788 of endothelin-1-induced contractions of the rabbit pulmonary artery, British Journal of Pharmacology, 113, 336-338

Furchgott, R.F., Zawadski, J.V. (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine, Nature, 288, 373-376

Fuxe, K., Kurosawa, M., Cintra, A., Goiny, M., Hallstrom, A., Rosen, L., Ungerstadt, U., Agnati, L. (1991) Endothelin-1 induced lesions in brain as a new model of focal ischaemia, Methods in Neurosciences, 17, 256-282

Fuxe, K., Kurosawa, N., Cintra, A., Hallstrom, A., Goiny, M., Rosen, L., Agnati, L.F., Ungerstedt, U. (1992) Involvement of local ischaemia in endothelin-1 induced lesions of the neostriatum of the anaesthetised rat, Experimental Brain Research, 88, 131-139.

Gaetani, P., Rodriguez y Baena, R., Grignani, G., Spanu, G., Pacchiarini, L., Paoletti, P. (1994) Endothelin and aneurysmal subarachnoid haemorrhage, a study of subarachnoid cisternal cerebrospinal fluid, Journal of Neurology Neurosurgery Psychiatry, 57, 66-72

Gandhi, C.R., Berkowitz, D.E., Watkins, W.D. (1994) Endothelins, biochemistry and pathophysiologic actions, Anaesthesiology, 80, 892-905

Gang-Zhi, W., Jin, Z., Shu-Li, S., Hon-Xi, A., Jin-Cheng, M., Lui, H-B. (1993) Increased plasma endothelin-1 concentration in patients with acute cerebral infarction and actions of endothelin-1 on pial arterioles of rat, Chinese Medical Journal, 106, 917-921

Garcia, J.L., Monge, L., Gomez, B., Dieguez, G. (1991) Response of canine cerebral-arteries to endothelin-1, Journal Of Pharmacy and Pharmacology, 43, 281-284

Gellai, M., Jugus, M., Fletcher, T., DeWolf, R., Nambi, P. (1994) Reversal of post-ischaemic acute renal failure with a selective ETA receptor antagonists in the rat. Journal of Clinical Investigation, 93, 900-906

Gellai, M., Jugus, M., Fletcher, T., Nambi, P., Ohlstein, E.H., Elliot, J.D. (1995) Nonpeptide endothelin receptor antagonists V. Prevention and reversal of acute renal failure in the rat by SB209670, Journal of Pharmacology and Experimental Therapeutics, 275, 200-206

Gennarelli, T.A., Thibault, L.E. (1982) Biomechanics of acute subdural haematoma, Journal of Trauma, 22, 680-686

Giaid, A., Gibson, S.J,, Ibrahim, N.B.N., Legon, S., Bloom, S.R., Yanagisawa, M., Masaki, T., Varndell, I.M., Polack, J.M. (1989) Endothelin-1 an endothelium derived peptide is expressed in neurones of the human spinal cord and dorsal root ganglia, Proceedings National Academy of Sciences U.S.A., 86, 7634-7638

Gill, R. (1994) The pharmacology of alpha-amino-3-hydroxy-5-methyl-isoxazole proprionate (AMPA)/Kainate antagonists and their role in cerebral ischaemia, Cerebrovascular and Brain Metabolism Reviews, 6, 225-256

Gill, R., Nordholm, L., Lodge, D. (1992) The neuroprotective actions of 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX) in a rat focal ischaemia model, Brain Research, 580, 35-43.

Gimbrone, M.A., Majeau, G.R., Atkinson, W.J., Sadler, W., Cruise, S.A. (1979) Angiotensin-converting enzyme activity in isolated brain microvessels, Life Sciences, 25, 1075-1084

Ginsberg, M.D., Prado, R., Dietrich, W.D., Busto, R. , Watson, B.D. (1987) Hyperglycaemia reduces the extent of cerebral infarction in rats, Stroke, 18, 570-574.

Giuffrida, R., Bellomo, M., Polizzi, G., Malatino, L.S. (1990) Ischaemia-induced changes in the immunoreactivity for endothelin and other vasoactive peptides in the brain of the mongolian gerbil, Journal of Cardiovascular Pharmacology, 20 (Suppl. 12), S41-S44.

Giuffrida, R., Malatino, L.S. (1992) Endothelin and transient cerebral ischaemia, An immunohistochemical study in the Mongolian gerbil, Brain Dysfunction, 5, 192-199.

Godfraind, T. (1993) Evidence for heterogeneity of endothelin receptor distribution in human coronary artery, British Journal of Pharmacology, 110, 1201-1205

Godfraind, T., Mennig, D., Morel, N., Wibo, M. (1989) Effect of endothelin-1 on calcium channel gating by agonists in vascular smooth muscle, Journal of Cardiovascular Pharmacology, 13 (Suppl. 5), S112-S117

Gotoh, O., Mohammed, A.A., McCulloch, J., Graham, D.I., Harper, A.M. , Teasdale, G.M. (1986) Nimodipine and the haemodynamic and histopathological consequences of middle cerebral artery occlusion in the rat, Journal of Cerebral Blood Flow and Metabolism, 6, 321-331.

Graham, D.I. (1988) Focal cerebral infarction, Journal of Cerebral Blood Flow and Metabolism, 8, 769-733.

Graham, D.I., Ford, I., Adams, J.H., Doyle, D., Teasdale, G.M., Lawrence, A., McLellan, D.R. (1989) Ischaemic brain damage is still common in fatal non-missile head injury, Journal of Neurology Neurosurgery and Psychiatry, 52, 346-359

Gray, G.A., Breu, V., Clozel, M. Endothelin receptors that modulate contraction of the rat fundus, Journal of Cardiovascular Pharmacology, (1995), 26 [Suppl. 3], S126-S129

Greenberg, D.A., Chan, J., Sampson, H.A. (1992) Endothelins and the nervous-system, Neurology, 42, 25-31

Gregory, P.C., Boisvert, D.P.J., Harper, A.M. (1980) Adenosine response on pial arterioles, influence of CO2 and blood pressure, Pflugers Archives, 386, 187-192

Grogaard, B., Schurer, L., Gerdin, B., Arfors, K.E. (1989) Delayed hypoperfusion after incomplete forebrain ischaemia in the rat, the role of polymorphonuclear leucocytes, Journal of Cerebral Blood Flow and Metabolism, 9, 500-505.

Grome, J..J, Harper, A.M. (1983) The effects of quipazine, a putative serotonin agonist on local cerebral blood flow and glucose utilisation in the rat and a pial

vascular diameter in the cat, Journal of Cerebral Blood Flow and Metabolism, 3 (Suppl. 1), S202

Gross, .PM., Teasdale, G.M., Graham, D.I. (1982) Intra-arterial histamine increases blood-brain transport in rats, American Journal of Physiology, 12, H307-H317

Gross, P.M., Beninger, R.J., Shaver, S.W., Wainman, D.S., Espinosa, F.J., Weaver, D.F. (1994) Metabolic and neuroanatomical correlates of barrel-rolling and oculoclonic convulsions induced by intraventricular endothelin-1, a novel peptidergic signalling mechanism in visuovestibular and oculomotor regulation?, Experimental Brain Research, 95, 397-408

Gross, P.M., Harper, A.M., Teasdale, G.M. (1981a) Cerebral circulation and histamine. II. Responses of pial veins and arterioles to receptor agonists, Journal of Cerebral Blood Flow and Metabolism, 1, 219-225

Gross, P.M., Teasdale, G.M., Angerson, W.J., Harper, A.M. (1981b) H2-receptors mediate increase in permeability of the blood-brain barrier during arterial histamine infusion, Brain Research, 210, 396-400

Gross, P.M., Weaver, D.F., Wainman, D.S., Chew, B.H., Espinosa, F.J., Nag, S. (1993) Potent metabolic stimulation of septal gray and cerebral white matter in vivo by intraventricular endothelin and nitric oxide, Biochemical and Biophysical Research Communications, 190 , 975-981

Grover, G.J., Dzwonczyk, S., Parham, C.S. (1993) The endothelin-1 receptor antagonist BQ-123 reduces infarct size in a canine model of coronary-occlusion and reperfusion, Cardiovascular Research, 27, 1613-1618

Grover, G.J., Sleph, P.G., Fox, M., Trippodo, N.C. (1992) Role of endothelin-1 and big endothelin-1 in modulating coronary vascular tone, contractile function and severity of ischaemia in rat hearts, Journal of Pharmacology and Experimental Therapeutics, 263, 1074-1082

Gu, X.H., Casley, D.J., Cincotta, M., Nayler, W.G. (1990) 125I endothelin binding to brain and cardiac membranes from normotensive and spontaneously hypertensive rats, European Journal of Pharmacology, 177, 205-209

Gulati, A. (1995) Endothelin mechanisms in the central nervous system: role in pathophysiology In Endothelin: role in health and disease ed. Gulati, A. Harwood Academic Publishers, Germany, pp 117-141

Gulbenkian, S., Wharton, J. (1993) Pattern of innervation of sensory Afferent systems in the circulation; immunocytochemistry and ultrastructure In, Vascular innervation and receptor mechanisms, new perspectives ed. Edvinsson L, Uddman R Academic press, London , pp 247-262

Hakim, A.M., Hogan, M..J, Carpenter, S. (1992) Time course of cerebral blood flow and histological outcome after focal cerebral ischaemia, Stroke, 23, 1138-1144.

Haller, C., Sercombe, R., Verrecchia, C., Fritsch, H., Seylaz, J., Kuschinsky, W. (1987) Effect of the muscarinic agonist carbachol on pial arteries in vivo after endothelial damage by air embolism, Journal Cerebral Blood Flow and Metabolism, 7, 605-611

Hamann, G., Isenberg, E., Strittmater, M., Schimerigk, K. (1993) Absence of elevation of big endothelin in subarachnoid haemorrhage Stroke, 24, 383-386

Hamel, E., Edvinsson, L., MacKenzie, E.T. (1988) Heterogeneous vasomotor responses of anatomically distinct feline cerebral arteries, British Journal of Pharmacology, 94, 423-436

Han, C., Abel, P.W. (1987) Neuropeptide Y potentiates contraction and inhibits relaxation of rabbit coronary arteries, Journal of Cardiovascular Pharmacology, 9, 675-681

Hansen, A.J. (1985) Effect of anoxia on ion distribution in the brain, Physiology Reviews, 65, 101-148.

Hardebo, J.E., Kahrstrom, J., Owman, C., Salford, L.G. (1989) Endothelin is a potent constrictor of human intracranial arteries and veins, Blood Vessels, 26, 249-253

Hardebo, J.E., Owman, C. (1990) Enzymatic barrier mechanisms for neurotransmitter monoamines and their precursors at the blood-brain barrier interface In, Pathophysiology of the blood-brain barrier ed. Johansson BB, Owman C, Widner H Elsevier, Amsterdam pp 41-55

Harper, A.M., MacKenzie, E.T. (1977) Effects of 5-hydroxytryptamine on pial arteriolar calibre in anaesthetised cats, Journal of Physiology, 271, 735.

Harris, R.J., Branston, N.M., Symon, L., Bayhan, M. , Watson, A. (1982) The effects of a calcium antagonist, nimodipine, upon physiological responses of the cerebral vasculature and its possible influence upon focal cerebral ischaemia, Stroke, 13, 759-766.

Hatashita, S., Hoff, J.T. (1986) Cortical tissue pressure gradients in early ischaemic brain oedema, Journal of Cerebral Blood Flow and Metabolism, 6, 1-7

Hay, D.W.P., Luttmann, M.A., Hubbard, W.C., Undem, B.J. (1993) Endothelin receptor subtypes in human and guinea-pig pulmonary tissues, British Journal of Pharmacology, 110, 1175-1183

Haynes, W.G., Strachan, F.E., Gray, G.A., Webb, D.J. (1995) Forearm vasoconstriction to endothelin-1 is mediated by ET(A) and ET(B) receptors in vivo in humans, Journal of Cardiovascular Pharmacology, 26 [Suppl. 3], S40-S43

Haynes, W.G., Webb, D.J. (1994) Contribution of endogenous generation of endothelin-1 to basal vascular tone. Lancet 344, 852-854

Heiss, W.D. (1983) Flow thresholds for functional and morphological damage of brain tissue, Stroke, 14, 329-331

Heiss, W.D., Hayakawa, T, Waltz, A.G. (1976) Cortical neuronal function during ischaemia, Archives of Neurology, 33, 813-820

Heiss,W.D. (1992) Experimental-evidence of ischaemic thresholds and functional recovery, Stroke, 23, 1668-1672

Heluy, V., Germain, G., Fournier, T., Ferre, F., Breuiller-Fouche, M. (1995) Endothelin ET(A) receptors mediate human uterine smooth muscle contraction, European Journal of Pharmacology, 285, 89-94

Herrling, P.L., Morris, R. , Salt, T.E. (1983) Effects of excitatory amino acids and their antagonists on membrane and action potentials of cat caudate neurones, Journal of Physiology, 339, 207-222.

Hickey, K.A., Rubanyi, G.M., Paul, R..J, Highsmith, R.F. (1985) Characterisation of a coronary vasoconstrictor produced by cultured endothelial cells American Journal of Physiology, 248, C550-C556

Higa, M., Davanipour, Z. (1991) Smoking and stroke, Neuroepidemiology, 10, 211-222

Hiley, C.R., Cowley, D.J., Pelton, J.T., Hargreaves, A.C. (1992) BQ-123, cyclo(-D-Trp-D-Asp-Pro-D-Val-Leu), is a non-competitive antagonist of the actions of endothelin-1 in SK-N-MC human neuroblastoma cells, Biochemical and Biophysical Research Communications, 184, 504-510

Hirata, Y., Emori, T., Eguchi, S., Kanno, K., Imai, T., Ohta, K., Marumo, F. (1993) Endothelin receptor subtype B mediates synthesis of nitric oxide by cultured bovine endothelial cells, Journal of Clinical Investigation, 91, 1367-1373

Hirata, Y., Fukuda, Y., Yoshimi, H., Emori, T., Shichiri, M., Marumo, F. (1989) Specific receptor for endothelin in cultured rat cardiocytes, Biochemical and Biophysical Research Communications, 160, 1438-1444

Hirata, Y., Matsunaga, T., Ando, K. (1990) Presence of plasma endothelin-1-like immunoreactivity in human cerebrospinal fluid, Biochemical and Biophysical Research Communications, 166, 1274-1278

Hirose H, Ide K, Sasaki T, Takahashi R, Kobayashi M, Ikemoto F, Yano M, Nishikibe M (1995) The role of endothelin and nitric oxide in modulation of normal and spastic cerebral vascular tone in the dog, European Journal of Pharmacology, 277, 77-87

Hisanaga, S., Hirokawa, N. (1990) Dephosphorylation-induced interactions of neurofilaments with microtubules, Journal of Biological Chemistry, 265, 21852-21858

Hokfelt, T., Post, C., Freedman, J., Lundberg, J.M., Terenius, L. (1989) Endothelin induced spinal lesion after intrathecal administration, Acta Physiologica Scandinavica, 137, 555-556

Hollenberg, S.M. (1993) Tachyphylaxis to the vasopressor effects of endothelin in rat aortic rings, American Journal of Physiology, 264, H352-H356

Hollmann, M., Hartley, M., Heinemann, S. (1991) Ca^{2+} permeability of KA-AMPA-gated glutamate receptor channels depends on subunit composition, Science, 252, 851-853.

Hom, G.J., Touhey, B., Rubanyi, G.M. (1992) Effects of intracoronary administration of endothelin in anaesthetised dogs: comparison with Bay K 8664 and U46619, Journal of Cardiovascular Pharmacology, 19, 194-200

Homma, S., Miyauchi, T., Sugishita, Y., Goto, K., Sato, M., Ohshima, N. (1992) Vasoconstrictor effects of endothelin-1 on myocardium microcirculation studied by the Langendorff perfusion method, differential sensitivities among microvessels, Microvascular Research, 43, 205-211

Hori, S., Kyotani, S., Inoue, S., Fukuda, K., Ohnishi, Y., Kusuhara, M., Aikawa, N., Yamaguchi, K., Nakamura, Y., Handa, S. (1991) Subepicardial microiscahemia formation induced by epicardial application of endothelin-1, Journal of cardiovascular Pharmacology, 17 (Suppl. 7), S300-S310

Horie, R., Yamori, Y., Hande, H. (1978) An essential difference between stroke prone SHR (SHRSP) and stroke resistant (SHRSR): quantitative evidence obtained by Yamori's preparation II, Japanese Heart Journal, 19, 630-632

Hossmann, K-A., Lechtape-Gruter H, Hossmann V (1973) The role of cerebral blood flow for the recovery of the brain after prolonged ischaemia, Z Neurology, 29, 375-384.

Hossmann, K.-A. (1991) Animal models of cerebral ischaemia. 1. Review of literature, Cerebrovascular Disease, 1, 2-15.

Hughes, A.K., Cline, R.C., Kohan, D.E. (1992) Alterations in renal endothelin-1 production in the spontaneously hypertensive rat, Hypertension, 20, 666-673

Hyman, E.S. (1961) Linear system for quantitating hydrogen at a platinum electrode, Circulation Research, 9, 1093-1097

Iadecola, C. (1992) Nitric oxide participates in the cerebrovasodilation elicited from cerebellar fastigial nucleus, American Journal of Physiology, 263, R1156-R1161

Iadecola, C., Pelligrino, D.A., Moskowitz, M.A., Lassen, N.A. (1994) Nitric oxide synthase inhibition and cerebrovascular regulation, Journal of Cerebral Blood Flow and Metabolism, 14, 175-192

Ibayashi, S., Ngai, A.C., Meno, J.R., Winn, H.R. (1991) Effects of topical adenosine analogs and forskolin on rat pial arterioles in vivo, Journal of Cerebral Blood Flow and Metabolism, 11, 72-76

Ibayashi, S., Ngai, A.C., Meno, J.R., Winn, H.R. (1988) The effects of dipyridamole and theophylline on rat pial vessels during hypocarbia, Journal of Cerebral Blood Flow and Metabolism, 8, 829-833

Ihara, M., Fukuroda, T., Saeki, T., Nishikibe, M., Kojiri, K., Suda, H., Yano, M. (1991) An endothelin receptor (ETA) antagonist isolated from streptomyces misakiensis, Biochemical and Biophysical Research Communications, 178, 132-137

Ihara, M., Noguchi, K., Saeki, T., Fukuroda, T., Tsuchida, S., Kimura, S., Fukani, T., Ishikawa, K., Nishikibe, M., Yano, M, (1992a) Biological profiles of highly potent novel endothelin antagonists for the ETA receptor, Life Sciences, 50, 247-255

Ihara, M., Saeki, T., Fukuroda, T., Kimura, S., Ozaki, S., Patel, A., Yano, M. (1992b) A novel radioligand [125I] BQ3020 selective for endothelin (ETB) receptors, Life Sciences, 51, 47-52

Iihara, K., Sasahara, M., Hashimoto, N., Uemura, Y., Kikuchi, H., Hazama, F. (1994) Ischaemia induces the expression of the platelet-derived growth factor-B chain in neurons and brain macrophages in vivo, Journal of Cerebral Blood Flow and Metabolism, 14, 818-824

Iijima, T., Mies, G. , Hossman, K.-A. (1992) Repeated negative DC deflections in rat cortex following middle cerebral artery occlusion are abolished by MK-801, effect on volume of ischaemic injury, Journal of Cerebral Blood Flow and Metabolism, 12, 727-733.

Inglis, F., Kuroda,Y., Bullock, R. (1992) Glucose hypermetabolism after acute subdural haematoma is ameliorated by a competitive NMDA antagonist, Journal of Neurotrauma, 9, 75-84

Inoue, A., Yanagisawa, M., Kimura, S., Kasuya, Y., Miyauchi, T. (1989) The human endothelin family, three structurally and pharmacologically distinct isopeptides

predicted by three separate genes, Proceedings of the National Academy of Sciences U.S.A, 86, 2863-2867

Ishikawa, K., Ihara, M., Noguchi, K., Mase, T., Mino, N., Saeki, T., Fukuroda, T., Fukami, T., Ozaki, S., Nagase, T., Nishikibe, M., Yano, M. (1994) Biochemical and pharmacological of a potent and selective endothelin-B receptor antagonist BQ788, Proceedings of the National Academy of Sciences U.S.A., 91, 4892-4896

Ishikawa, T., Yanagisawa, M., Kimura, S., Goto, K., Masaki, T. (1988) Positive inotropic action of novel vasoconstrictor peptide endothelin on guinea pig atria, American Journal of Physiology, 255, H970-H977

Ito, U., Reulen, H.J., Tomita, H., Ikeda, J., Saito, J., Maehara, T. (1988) Formation and propagation of brain oedema fluid around human brain metastases. A CT study, Acta Neurochirurgica, 90, 35-41

Itoh, S., Sasaki, T., Asai, A., Kuchino, Y. (1994) Prevention of delayed vasospasm by an endothelin ETA receptor antagonist BQ123, change of ETA receptor mRNA expression in a canine subarachnoid haemorrhage model, Journal of Neurosurgery, 81, 759-764

Itoh, S., Sasaki, T., Ide, K., Ishikawa, K., Nishikibe, M., Yano, M. (1993) A novel endothelin ETA receptor antagonist BQ485 and its preventive effect on experimental cerebral vasospasm in dogs, Biochemical and Biophysical Research Communications, 195, 969-975

Jacewicz, M., Brint, S., Tanabe, J. , Pulsinelli, W.A. (1990a) Continuous nimodipine treatment attenuates cortical infarction in rats subjected to 24 hours of focal cerebral ischaemia, Journal of Cerebral Blood Flow and Metabolism, 10, 89-96.

Jacewicz, M., Brint, S., Tanabe, J., Wang, X-J. , Pulsinelli, W.A. (1990b) Nimodipine pretreatment improves cerebral blood flow and reduces brain edema in conscious rats subjected to focal cerebral ischaemia, Journal of Cerebral Blood Flow and Metabolism, 10, 903-913.

Jaffer, F.E., Knauss, T.C., Poptic, E., Abboud, H.E. (1990) Endothelin stimulates PDGF secretion in cultured human mesangial cells, Kidney International, 38, 1193-1198

Jamieson, K.G., Yelland, J.D.N. (1972) Surgically treated traumatic subdural haematoma, Journal of Neurosurgery, 37, 137-149

Janat, M.F., Argraves, W.S., Liau, G. (1992) Regulation of vascular smooth muscle cell integrin expression by transforming growth factor beta1 and by platelet-derived growth factor-BB, Journal of Cellular Physiology, 151, 588-595

Jansen, I., Alafaci, C., McCulloch, J., Uddman, R., Edvinsson, L. (1991) Tachykinins (substance P, neurokinin A, neuropeptide K, neurokinin B) in the cerebral circulation, vasomotor responses in vitro and in situ, Journal of Cerebral Blood Flow and Metabolism, 11, 567-575

Jansen, I., Fallgren, B., Edvinsson, L. (1989) Mechanisms of actions of endothelin on isolated feline cerebral arteries, in vitro pharmacology and electrophysiology, Journal of Cerebral Blood Flow and Metabolism, 9, 743-747

Jansen, I., Nilsson, L., Edvinsson, L. (1993) Calcitonin gene-related peptide and blood vessels In, Vascular innervation and receptor mechanisms, new perspectives ed. Edvinsson L, Uddman R Academic press, London pp 263-279

Jones, C., Hiley, C., Pelton, J., Mohr, M. (1989) Autoradiographic visualisation of the binding sites for 125I-Endothelin in rat and human brain, Neuroscience Letters, 97, 276-279

Jones, T.J., Morawetz, R.B., Crowell, R.M., Marcoux, F.W., Fitzgibbon, S.J., Degirolami, U. , Ojemann, R.G. (1981) Thresholds of focal cerebral ischaemia in awake monkeys, Journal of Neurosurgery, 54, 773-782.

Kadel, K.A., Heistad, D.A., Faraci, F.M., (1990) Effects of endothelins on blood vessels of the brain and the choroid plexus, Brain Research, 518, 78-82

Kagstrom, E., Smith, M.-L. , Siesjö, B.K. (1983) Recirculation in the rat brain following incomplete ischaemia, Journal of Cerebral Blood Flow and Metabolism, 3, 183-192.

Kamitani, T., Little, M.H., Ellis, E.F. (1985) Evidence for a possible role of the brain kallikrein-kinin system in the modulation of the cerebral circulation, Circulation Research, 57, 545-552

Kapadia, S.E., de Lanerolle, N.C. (1984) Immunohistochemical and electron microscopic demonstration of vascular innervation in the mammalian brain stem, Brain Research, 292, 33-39

Kaplan, B., Brint, S., Tanabe, J., Jacewicz, M., Wang, X-J., Pulsinelli, W. (1991) Temporal thresholds for neocortical infarction in rats subjected to reversible focal cerebral ischaemia, Stroke, 22, 1032-1039.

Karaki, H., Sudwarjo, S.A., Hori, M., Sakata, K., Urade, Y., Takai, M., Okada, T. (1993) ETB receptor antagonist IRL1038 selectively inhibits the endothelin induced endothelium dependent vascular relaxation, European Journal of Pharmacology, 231, 371-374

Karne, S., Jayawickreme, C.K., Lerner, M.R., (1993) Cloning and characterisation of an endothelin-3 specific receptor (ETC receptor) from Xenopus Laevis dermal melanocytes, Journal of Biological Chemistry, 268, 19126-19133

Karnushina, I.L., Palacios, J.M., Barbin, G. (1980) Studies on a capillary-rich fraction isolated from brain: Histaminic components and characterisation of the histamine receptors linked to adenylate cyclase, Journal of Neurochemistry, 34, 1201-1208

Kase, C.S., Mohr, J.P., Caplan, L.R. (1992) Intracerebral haemorrhage In, Stroke, pathophysiology, diagnosis and management eds. Barnett HJM, Mohr JP, Stein BM, Yatsu FM Churchill Livingstone Inc., New York pp 561-615

Kauser, K., Rubanyi, G.M., Harder, D.R. (1990) Endothelium dependent modulation of endothelin induced vasoconstriction and membrane depolarisation in cat cerebral arteries, Journal of Pharmacology and Experimental Therapeutics, 252, 93-97

Kazda, S., Garthoff, B., Krause, H.P., Schlossmann, K. (1982) Cerebrovascular effects of the calcium channel antagonistic dihydropyridine derivative nimodipine in animal experiments, Arzneimittell Forrschung / Drug Research, 32, 331- 338.

Kelly, R.A., Eid, H., Kramer, B.K., O'Neill, M., Laing, B.T., Reers, M., Smith, T.W. (1990) Endothelin enhances the contractile responsiveness of adult rat ventricular myocytes to calcium by a pertussis toxin sensitive pathway, Journal of Clinical Investigation, 86, 1164-1171

Kety, S.S. (1951) The theory and applications of the exchange of inert gas at the lungs and tissues, Pharmacology Reviews, 3, 1-41.

Kety, S.S., Schmidt, C.F. (1945) The determination of cerebral blood flow in man by use of nitrous oxide in low concentration, American Journal of Physiology, 143, 53-66

Kiowski, W., Sutsch, G., Hunziker, P., Muller, P., Kim, J., Oechslin, E., Schmitt,R., Jones, R., Bertel, O. (1995) Evidence for endothelin-1-mediated vasoconstriction in severe chronic heart failure, Lancet, 346, 732-736

Kirino, T. (1982) Delayed neuronal death in the gerbil hippocampus following ischaemia, Brain Research, 239, 57-69.

Kirino, T., Sano, K. (1984) Selective vulnerability in the gerbil hippocampus follwing transient ischaemia, Acta Neuropathologica, 62, 210-208

Kirino, T., Tamura, A., Sano, K. (1984) Neuronal death in the rat hippocampus following transient forebrain ischaemia, Acta Neuropathologica, 64, 139-147

Kissinger, P.T., Park, J.B., Adams, R.N. (1973) Voltametry in brain tissue, a new neurophysiologic measurement, Brain Research, 55, 209-213

Kitazono, T., Heistad, D.D., Faraci, F.M. (1993) Activation of endothelin B receptors produces dilatation of the basilar artery in vivo, Circulation, 88 (Suppl), I-170 (ABSTRACT)

Kloog, Y., Sokolovsky, M. (1989) Similarities in mode and sites of action of sarafotoxins and endothelins, Trends in Pharmacological Sciences, 10, 212-214

Klugman, K.P., Lembeck, F., Markowitz, S., Mitchell, G., Rosendorff, D. (1980) Substance P increases hypothalamic blood flow via an indirect adrenergic-cholinergic interaction, British Journal of Pharmacology. 71, 623-629

Kobari, M., Fukuuchi, Y., Tomita, M., Tanahashi, N., Konno, S., Takeda, H. (1994a) Dilatation of cerebral microvessels mediated by endothelin ETB receptor and nitric oxide in cats, Neuroscience Letters, 176, 157-160

Kobari, M., Y. Fukuuchi, M. Tomita, N. Tanahashi, S. Konno, H. Takeda, (1994b) Constriction/dilatation of the cerebral microvessels by intravascular endothelin-1 in cats, Journal Cerebral Blood Flow and Metabolism, 14, 64-72

Kobayashi, H., Hayashi, M., Kobayashi, S., Kabuto, M., Handa, Y., Kawano, H., Ide, H. (1991) Cerebral vasospasm and vasoconstriction caused by endothelin, Neurosurgery, 28, 673-679

Kohno, M., Ikeda, M., Johchi, M., Horio, T., Yasunari, K., Kurihara, N., Takeda, T. (1993) Interaction of PDGF and natriuretic peptides on mesangial cell proliferation and endothelin secretion, American Journal of Physiology, 265, E673-E679

Kohzuki, M., Johnston, C.I, Chai, S.Y. Casley. D.J., Mendelsohn, F.A. (1989) Localisation of endothelin receptors in rat kidney, European Journal of Pharmacology, 160, 193-194

Kontos, H., Wei, E.P., Povlishock, J.T., Christman, C.W. (1984) Oxygen radicals mediate the cerebral arteriolar dilatation from arachidonic acid and bradykinin in cats, Circulation Research, 55, 295-303

Kontos, H.A. (1980) Response to Kuschinsky and Wahl, Microvascular Research, 19, 387-388

Kontos, H.A., Wei, E.P., Ellis, E.F. (1980) Induction of endothelial lesions in pial arterioles by arachidonic acid and prostaglandin G, Stroke, 11, 129-135

Kontos, H.A., Wei, E.P., Navari, R.M., Levasseur, J.E., Rosenblum, W.I., Patterson, J.L. (1978) Responses of cerebral arteries and arterioles to acute hypotension and hypertension, American Journal of Physiology, 234, H371-H383

Kontos, H.A., Wei, E.P., Povlishock, J.T. (1980) Cerebral arteriolar damage by arachidonic acid and prostaglandin G2, Science, 209, 1242-1245

Korchak, D.M., Gysling, K., Benfield, S (1985) The subcellular distribution of peptide histidine isoleucine amide - 27 like peptide in rat brain and their release from rat cerebral cortical slices in vitro, Journal of Neurochemistry, 44, 255-259

Koseki, C., Imai, M., Hirata, Y., Yanagisawa, M., Masaki, T. (1989) Autoradiographic distribution in rat tissue of binding sites for endothelin, a neuropeptide?, American Journal of Physiology, 256, R858-R866

Koudstaal, P.J. (1993) Cardioembolic stroke In, Current review of cerebrovascular disease eds. Fisher, M., Bogousslavsky, J. Current Medicine Inc., Philadelphia pp 41-47

Kourembanas, S., McQuillan, L.P., Leung, G.K., Faller, D.V. (1993) Nitric Oxide regulates the expression of vasoconstrictors and growth factors by vascular endothelium under both normoxia and hypoxia, Journal Clinical Investigation, 92, 99-104

Kraus, G.E., Bucholz, R.D., Yoon, K-W., Knuepfer, M.M., Smith, K.R. (1991) Cerebrospinal fluid endothelin-1 and endothelin-3 levels in normal and neurosurgical patients, a clinical study and literature review, Surgical Neurology, 35, 20-29

Krause, D.N., Edvinsson, L. (1984) Pharmacological characterisation of cerebrovascular cholinergic receptors, combined biochemical and physiological approach Monogram of Neural Sciences, 11, 51-57

Kuchan, M.J., Frangos, J.A. (1993) Shear stress regulates endothelin-1 release via protein kinase C and cGMP in cultured endothelial cells, American Journal of Physiology, 264, H150-H156

Kurihara, H., Ochi, A., Kawaguchi, T., Niwa, M., Kataoka, Y., Mori, K. (1990) Localisation and characterisation of endothelin receptors in human gliomas, a growth factor?, Neurosurgery 27, 275-281

Kurihara, H., Yoshizumi, M., Sugiyama, T., Takaku, F., Yanagisawa, M., Masaki, T., Hamaoki, M., Kato, H., Yazaki, Y. (1989) Transforming Growth Factor - ß stimulates the expression of endothelin mRNA by vascular endothelial cells, Biochemical and Biophysical Research Communications, 159, 1435-1440

Kuroda, Y., Bullock, R., Selman, W.R. (1992a) Local cerebral blood flow mapping before and after removal of acute subdural haematoma in the rat, Neurosurgery, 30, 687-691

Kuroda, Y., Fujisawa, H., Strebel, S., Graham, D.I., Bullock, R., Marmarou, A., Young, W. (1994) Effect of neuroprotective N-methyl-D-aspartate antagonists on increased intracranial pressure: Studies in the rat acute subdural haematoma model, Neurosurgery, 35, 106-112

Kuroda,Y., Bullock, R., Selman, W., Marmarou, A. (1992b) Failure of cerebral blood flow-metabolism coupling after acute subdural haematoma in the rat, Neurosurgery, 31, 1062-1071

Kurumaji, A., Nehls, D.G., Park, C.K. , McCulloch, J. (1989) Effects of NMDA antagonists, MK-801 and CPP, upon local cerebral glucose use, Brain Research, 496, 268-284.

Kuschinsky, W. , Wahl, M. (1978) Local chemical and neurogenic regulation of cerebral vascular resistance, Physiology Reviews, 58, 656-689.

Kuschinsky, W., Wahl, M. (1975) Alpha receptor stimulation by endogenous and exogenous norepinephrine and blockade by phentolamine in pial arteries of cats, Circulation Research, 37, 168-174

Kuschinsky, W., Wahl, M. (1979) Perivascular pH and pial arterial diameter during bicucculine induced seizures in cats, Pflugers Archives, 382, 81-85

Kuschinsky, W., Wahl, M. (1980) Letter to editor, Microvascular Research, 19, 385-386

Kuschinsky, W., Wahl, M., Bosse, O., Thurau, K. (1972) Perivascular potassium and pH as determinants of local pial arterial diameter in cats, Circulation Research, 31, 240-247

Kuschinsky, W., Wahl, M., Neiss, A. (1974) Evidence for cholinergic dilatory receptors in pial arteries of cats, Pflugers Arch, 347, 199-202

La Douceur, D.M., Flynn, M.A., Keiser, J.A., Reynolds, E., Haleen, S.J. (1993) ET(A) and ET(B) receptors coexist on rabbit pulmonary artery vascular smooth muscle mediating contraction, Biochemical and Biophysical Research Communications, 196, 209-215

Ladenheim. R., Lacroix. I., Foignant-Chaverot, N., Strosberg, A., Couland, P. (1993) Endothelins stimulate c-fos and nerve growth factor expression in astrocytes and astrocytoma, Journal of Neurochemistry, 60260-266

Lago, M.A., Cousins, R.D., Gao, A., Leber, J.D., Peishoff, C.E., Ohlstein, E.H., Elliott, J.D. Rational design and synthesis of potent non peptide ET receptor antagonists (abstract no. C44) 4th International conference on Endothelin, (1995) 23-26 April; London U.K.

Landau, W.M., Freygang, W.H., Rowland, L.P., Sokoloff, L., Kety, S.S. (1955) The local circulation of the brain: value in the unanaesthetised and anaesthetised cat, Transactions of the American Neurology Association, 80, 125-129

Lattera J.J., Goldstein G.W. (1992) The blood-brain barrier in vitro and in vivo In, Physiology and pharmacology of the blood-brain barrier ed. Bradbury MWB Springer Verlag, Berlin pp 417-438

Lawrence, E., Brain, S.D. (1993) Big endothelin-1 and big endothelin-3 are constrictor agents in the microvasculature, Evidence for the local phosphoramidon-sensitive conversion of big endothelin-1, European Journal of Pharmacology, 233, 243-250

Le Peillet, E., Arvin, B., Moncada. C. , Meldrum, B.S. (1992) The non-NMDA antagonists, NBQX and GYKI 52466, protect against cortical and striatal cell loss following transient global ischaemia in the rat, Brain Research, 571, 115-120.

Lechleitner, .P, Genser, N., Mair, J., Maier, J., Artner-Dworzak, E., Dienstl, F., Puschendorf, B. (1992) Endothelin-1 in patients with complicated and uncomplicated myocardial infarction, Clinical Investigator, 70, 1070-1072

Lee, M.E., Delamonte, S.M., Ng, S.C., Bloch, K.D., Quertermous, T. (1990) Expression of the potent vasoconstrictor endothelin in the human central nervous system, Journal of Clinical Investigation, 86, 141-147

Lee, T.J.F., Hume, W.R., Su, C., Bevan, J.A. (1978a) Neurogenic vasodilation of cat cerebral arteries, Circulation Research, 42, 535-540

Lee, T.J.F., Saito, A., Berezin, I. (1978b) Vasoactive intestinal polypeptide-like substance, the potential transmitter for cerebral vasodilatation, Science, 224, 898-901

Leenders, K.L., Wolfson, L., Gibbs, J.M. (1985) The effects of L-DOPA on regional cerebral blood flow and oxygen metabolism in patients with Parkinson's disease, Brain, 108, 171-191

Lembeck, F., Gamse, R. (1982) Substance P in peripheral sensory processes In, Substance P in the nervous system, CIBA Foundation Symposium 91 Pittman, London, pp 35-54

Lerman ,A., Kubo, S.H., Tschumperlin, L.K., Burnett, J.C. (1992) Plasma endothelin concentrations in humans with end-stage heart failure and after heart transplantation, Journal of the American College of Cardiology, 20, 849-853

Levin ER, Issackson PJ, Hu RM (1992) Endothelin receptors on cultured fetal rat diencephalic glia Journal Neurochemistry 58, 659-666

Li, H. , Buchan, A.M. (1993) Treatment with an AMPA antagonist 12 hours following severe normothermic forebrain ischaemia prevents CA_1 neuronal injury, Journal of Cerebral Blood Flow and Metabolism, 13, 933-939.

Lin, W.W., Lee, C.Y. (1990) Biphasic effects of endothelin in the guinea pig ileum, European Journal of Pharmacology, 176, 57-62

Lin, W.W., Lee, C.Y. (1992) Intestinal relaxation by endothelin isopeptides, involvement of Ca2+ activated K+ channels, European Journal of Pharmacology, 219, 355-360

Linton-Dahlof P, Dahlof C (1993) Noradrenaline and neuropeptide Y in the sympathetic system, pre-junctional interactions and regulation of release In, Vascular innervation and receptor mechanisms, new perspectives ed. Edvinsson L, Uddman R Academic press, London pp 159-173

Liu, J., Chen, R., Casley, D.J., Nayler, W.G. (1990) Ischaemia and reperfusion increase 125I labelled endothelin-1 binding in rat cardiac membranes, American Journal of Physiology, 258, 829-835

Lopez-Farre, A., Gomez-Garre, D., Bernabeu F, Lopez-Novoa, J.M. (1991) A role for endothelin in the maintenance of post ischaemic renal failure in the rat, Journal of Physiology, 444, 513-522.

Lucas, D.R. , Newhouse, J.P. (1957) The toxic effect of sodium L-glutamate on the inner layers of the retina, Archives of Opthalmology, 158, 193-201.

Lucher, T.F. (1991) Endothelin, Journal of Cardiovascular Pharmacology, 18 (Suppl. 10), S15-S22

Luscher, T.F., (1993) Do we need endothelin antagonists?, Cardiovascular Research, 27, 2089-2093

Lustig, H.S., Chan, J., Greenberg, D.A. (1992) Comparative neurotoxic potential of glutamate endothelins and platelet activating factor in cerebral cortical cultures, Neuroscience Letters, 139, 15-18

Lysko, P.G., Feuerstein, G.Z., Pullen, M., Wu, H-L., Nambi, P. (1991) Identification of endothelin receptors in cultured cerebellar neurones, Neuropeptides, 18, 83-86

MacArthur, H., Warner, T.D., Wood, E.G., Corder, R., Vane, J.R. (1994) Endothelin-1 release from cells in culture is elevated both acutely and chronically by short periods of mechanical stretch, Biochemical and Biophysical Research Communications, 200, 395-400

MacCumber, M., Ross, C., Snyder, S. (1990) Endothelin in brain, receptor, mitogenesis and biosynthesis in glial cells, Proceedings of the National Academy of Sciences U.S.A, 87, 2359-2363.

Mackay, K.B., Patel, T.R., Galbraith, S.L., Woodruff, G.N., McCulloch, J. (1996) The relationship between glutamate and cerebral blood flow after focal cerebral ischaemia in the cat: effect of pre-treatment with enadoline (a kappa receptor agonist), Brain Research (in press)

Mackay, K.B., Kusumoto, K., Graham, D.I. , McCulloch, J. (1993a) Effect of the kappa-1 opioid agonist CI-977 on ischaemic brain damage and cerebral blood flow after middle cerebral artery occlusion in the rat, Brain Research, 629, 10-18.

Mackay, K.B., Kusumoto, K., Graham, D.I. , McCulloch, J. (1993b) Focal cerebral ischaemia in the cat, pretreatment with a kappa-1 opioid receptor agonist, CI-977, Brain Research, 618, 213-219.

MacKenzie, E.T., Scatton, B. (1987) Cerebral circulatory and metabolic effects of perivascular neurotransmitters, CRC Critical Reviews in Clinical Neurobiology, 2, 357-419

MacKenzie, E.T., Farrar, J.K., Fitch, W., Graham, D.I., Gregory, P.C., Harper A.M. (1979) Effects of haemorrhagic hypotension on the cerebral circulation I. Cerebral blood flow and pial arteriolar calibre, Stroke, 10, 711-727

MacKenzie, E.T., Strandgaard, S., Graham, D.I., Jovers, J.V., Harper A.M., Farrar, J.K. (1976) Effects of acutely induced hypertension in cats on pial arteriolar calibre,

local blood cerebral blood flow and the blood-brain barrier, Circulation Research, 39, 33-41

Macrae, I.M., Robinson, M., McAuley, M., Reid, J., McCulloch, J. (1991) Effects of intracisternal endothelin-1 injection on blood flow to the lower brain stem, European Journal of Pharmacology, 203, 85-91

Macrae, I.M., Robinson, M.J., Graham, D.I., Reid, J.L., McCulloch, J. (1993) Endothelin-1-induced reductions in cerebral blood flow, dose dependency, time course and neuropathological consequences, Journal of Cerebral Blood Flow and Metabolism, 13, 276-287

Maguire, J.J., Davenport, A.P. (1995) ET(A) receptor-mediated constrictor responses to endothelin peptides in human blood vessels in vitro, British Journal of Pharmacology, 115, 191-197

Maguire, J.J., Kuc, R.E., Doherty, A.M., Davenport, A.P. (1995) Potency of PD155080, an orally active ET(A) receptor antagonist, determined for human endothelin receptors, Journal of Cardiovascular Pharmacology, 26 [Suppl. 3], S362-S364

Malek, A., Izumo, S. (1992) Physiological fluid shear stress causes the down regulation of ET-1 mRNA in bovine aortic endothelium, American Journal of Physiology, 263, C389-C396.

Manbe K, Shirahase H, Usui H, Kurahashi K, Fujiwara H (1989) Endothelium dependent contractions induced by angiotensin I and angiotensin II in canine cerebral artery Journal of Pharmacology and Experimental Therapeutics 251, 317-320

Marletta, M.A. (1993) Nitric oxide synthase structure and mechanism, Journal of Biological Chemistry, 268, 12231-12234

Marsault, R., Feolde, E., Frelin, C. (1993) Receptor externalisation determines sustained contractile responses to endothelin-1 in the rat aorta. American Journal of Physiology 264, C687-C693

Marsault, R., Vigne, P., Breittmayer, J-P., Frelin, C. (1990) Astrocytes are targets for endothelins and sarafotoxins, Journal of Neurochemistry, 54, 2142-2144

Marshall, J.J., Wei, E.P., Kontos, H.A. (1988) Independent blockade of cerebral vasodilatation from acetylcholine and nitric oxide, American Journal of Physiology, 255, H847-H854

Martin de Aguilera, E., Irurzuni, A., Vila, J.M., Aldasoro, M., Galeote, M.S., Lluch, S. (1990) Role of endothelium and calcium channels in endothelin induced contraction of human cerebral arteries, British Journal of Pharmacology, 99, 439-440

Masaki, T., Vane, J., Vanhoutte, P.M. (1994) International Union of Pharmacology Nomenclature of Endothelin Receptors, Pharmacology Reviews, 46, 137-142 .

Matsumoto, H., Suzuki, N., Onda, H., Fujino, M. (1989) Abundance of endothelin-3 in rat intestine, pituitary gland and brain, Biochemical and Biophysical Research Communications, 164, 74-80

Matsumoto, K., Graf, R., Rosner, G., Shimada, N. , Heiss, W.-D. (1992) Flow thresholds for extracellular purine catabolite elevation in cat focal ischaemia, Brain Research, 579, 309-314.

Matsumoto, K., Graf, R., Rosner, G., Taguchi, J. , Heiss, W.-D. (1993) Elevation of neuroactive substances in the cortex of cats during prolonged focal ischaemia, Journal of Cerebral Blood Flow and Metabolism, 13, 586-594.

McAuley, M.A., Patel, T.R., Galbraith, S., McCulloch, J. (1994) Endothelin and its pathophysiological role in the cerebral circulation. In, Pharmacology of Cerebral Ischaemia (Krieglstein, J., Oberpichler-Schwenk, H., eds.), Wissenschaftliche Verlagsgesellschaft, Stuttgart, pp 511-524.

McBurney, R.N., Neering, I.R. (1987) Neuronal calcium homeostasis, Trends in Neurosciences, 10, 164-169

McCarron, R.M., Wang, L., Stanimirovic, D.B., Spatz, M. (1993) Endothelin induction of adhesion molecule expression on human brain microvascular endothelial cells, Neuroscience Letters 156, 31-34

McCulloch, J., Edvinsson, L. (1980) Cerebral circulatory and metabolic effects of vasoactive intestinal polypeptide, American Journal of Physiology, 238, H449-H456

McCulloch, J., Edvinsson, L. (1984) Cerebrovascular smooth muscle reactivity, a critical appraisal of in vitro and in situ techniques, Journal of Cerebral Blood Flow Metabolism, 4, 129-139

McCulloch, J., Edvinsson, L. (1987) Calictonin gene-related peptide and the trigeminal innervation of the cerebral circulation In, Peptidergic mechanisms in the cerebral circulation ed. Edvinsson L., McCulloch J. VPH Verlasgesellschaft, Weinheim pp 132-151

McCulloch, J., Kelly, P.A.T., Uddman, R., Edvinsson, L. (1983) Functional role for vasoactive intestinal polypeptide in the caudate nucleus, 2- Deoxy [14C] glucose investigations, Proceedings of the National Academy of Sciences U.S.A., 80, 1472-1476

McCulloch, J., Uddman, R., Kingman, T., Edvinsson, L. (1986) Calcitonin gene related peptide, functional role in cerebrovascular regulation, Proceedings of the National Academy of Sciences. USA, 83, 5731-5735

McCulloch, J., Bullock, R. , Teasdale, G.M. (1991) Excitatory amino acid antagonists, opportunities for the treatment of ischaemic brain damage in man; In Excitatory Amino Acid Antagonists, ed. Meldrum, B.S, Blackwell, pp. 287-325.

McCulloch, J., Edvinsson, L., Watt, P. (1982a) Comparison of the effects of potassium and pH on the calibre of cerebral veins and arteries, Pflugers Archives, 393, 95-98

McCulloch, J., Kelly, P.A.T. , Ford, I. (1982b) Effect of apomorphine on the relationship between local cerebral glucose utilisation and local cerebral blood flow (with an appendix on its statistical analysis), Journal of Cerebral Blood Flow and Metabolism, 2, 487-499.

McCulloch, K., MacLean, M.R. (1995) Endothelin B receptor mediated contraction of human and rat pulmonary resistance arteries and the effect of pulmonary hypertension endothelin responses in the rat, Journal of Cardiovascular Pharmacology, 26 (Suppl. 13), S169-S176

Mchedlishvili, G., Kuridze, N. (1984) The modular organisation of the pial arterial system in phylogeny, Journal of Cerebral Blood Flow and Metabolism, 4, 391-396.

McMahon, E.G., Palomo, M.A., Moore, W.M. (1991) Phosphoramidon blocks pressor activity of big endothelin (1-39) and lowers blood pressure in spontaneously hypertensive rats, Journal of Cardiovascular Pharmacology, 17 (Suppl. 17), S29-S33

McMurdo, L., Corder, R., Thiemermann, C., Vane, J.R. (1993) Incomplete inhibition of the pressor effects of endothelin-1 and related peptides in the anaesthetised rat with BQ-123 provides evidence for more than one vasoconstrictor receptor, British Journal of Pharmacology, 108, 557-61

McMurdo, L., Thiemermann, C., Vane, J.R. (1994) The effects of the endothelin ET(A) receptor antagonist, FR139317, on infarct size in a rabbit model of acute myocardial ischaemia, British Journal of Pharmacology, 112, 75-80

Meija, J.A., Pernow, J., von Holst, H., Rudehill, A., Lundberg, J.M. (1988) Effects of neuropeptide Y, calcitonin gene-related peptide, substance P and capsaicin on cerebral arteries in man and animals, Journal of Neurosurgery, 69, 913-918

Meldrum, B. (1990) Protection against ischaemic neuronal damage by drugs acting on excitatory neurotransmission, Cerebrovascular and Brain Metabolism Reviews, 2, 27-57

Meldrum, B.S., Swan, J.H., Leach, M.J., Millan, M.H., Gwinn, R., Kadota, K., Graham, S.H., Chen, J. , Simon, R.P. (1992) Reduction of glutamate release and protection against ischaemic brain damage by BW 1003C87, Brain Research, 593, 1-6.

Mendelow, A.D., Eidelman, B.H., McCalder, T.A., Rosendorff, C. (1977) Cerebrovascular response to infused 5-Hydroxytryptamine in the baboon I. 5-Hydroxytryptamine infusion, Stroke, 8, 322-329

Mendelow, A.D. (1993) Mechanisms of ischaemic brain damage with intracerebral hemorrhage, Stroke, 24, I115-I117

Mies, G., Ishimaru, S., Xie, Y., Seo, K. , Hossmann, K.-A. (1991) Ischaemic thresholds of cerebral protein synthesis and energy state following middle cerebral artery occlusion in the rat, Journal of Cerebral Blood Flow and Metabolism, 11, 753-761.

Miller, C., Lampard, D.G., Alexander, K., Brown, W.A. (1980) Local cerebral blood flow following transient cerebral ischaemia I. Onset of impaired reperfusion within the first hour following global ischaemia, Stroke, 11,534-541.

Miller, J.D., Bullock, R., Graham, D.I., Chen, M.-H., Teasdale, G.M. (1990) Ischaemic brain damage is a model of acute subdural haematoma, Neurosurgery, 27, 433-439

Miller, R.J. (1991) The control of neuronal Ca^{2+} homeostasis, Progress in Neurobiology, 37, 255-285.

Mima, T., Yanagisawa, M., Shigeno, T., Saito, A., Goto, K., Takakura, K., Masaki, T. (1989) Endothelin acts in feline and canine cerebral arteries from the adventitial side, Stroke, 20, 1553-1556

Minanisawa, H., Nordström, C.-H., Smith, M.-L. , Siesjö, B.K. (1990) The influence of mild body and brain hypothermia in ischaemic brain damage, Journal of Cerebral Blood Flow and Metabolism, 10, 365-374.

Mino, N., Kobayashi, M., Nakajima, A., Amano, H., Shimamoto, K., Watanabe, K., Nishikibe, M., Yano, M., Ikemoto, F. (1992) Protective effect of a selective endothelin receptor antagonist BQ-123 in ischaemic acute renal failure in rats, European Journal of Pharmacology, 221, 77-83

Misrahy, G.A., Clark, L.C. (1956) Use of platinum black cathode for local blood flow measurements in vivo, Proceedings of the International Congress of Physiology, 20, 650

Mohammed, A.A., Gotoh, O., Graham, D.I., Osborne, K.A., McCulloch, J., Mendelow, A.D., Teasdale, G.M., Harper, A.M. (1985) Effect of pretreatment with the calcium antagonist nimodipine on local cerebral blood flow and metabolism and histopathology after middle cerebral artery occlusion, Annals of Neurology, 18, 705-711.

Mohr, J.P., Kistler, J.P., Fink, M.E. (1992) Intracranial aneurysms In, Stroke, pathophysiology, diagnosis and management eds. Barnett HJM, Mohr JP, Stein BM, Yatsu FM Churchill Livingstone Inc., New York pp 617-643

Mohr, J.P., Sacco, R.L. (1992) Classification of ischaemic strokes In, Stroke, pathophysiology, diagnosis and management eds. Barnett HJM, Mohr JP, Stein BM, Yatsu FM Churchill Livingstone Inc., New York pp 271-283

Mokry, M., Ishiyama, N., Kleinert, R., Auer, L.M. (1987) Nimodipine opens collaterals during acute focal ischaemia, Journal of Cerebral Blood Flow and Metabolism, 7 (Suppl. 1), S164

Mokry, M., Sakaki, T., Kleinert, R., Mies, G., Pucher, R.K., Auer, L.M. (1989) Focal ischaemia by temporary MCA occlusion in the cat: influence of nimodipine pretreatment on ischaemic cell volume, Journal of Cerebral Blood Flow and Metabolism, 9 (Suppl. 1), S314

Molenaar, P., O'Reilly, G., Sharkey, A., Kuc, R.E., Harding, D.P., Plumpton, C., Gresham, G.A., Davenport, A.P. (1993) Characterisation and localisation of endothelin receptor subtypes in the human atrioventricular conducting system and myocardium, Circulation Research, 71, 526-538

Moncada, S., Gryglewski, R., Bunting, S., Vane, J.R. (1976) An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation, Nature, 263, 663-665

Moncada, S., Palmer, R.M.J., Higgs, E.A. (1991) Nitric oxide, physiology, pathophysiology and pharmacology, Pharmacology Reviews, 43, 109-142

Moravec, C.S., Reynolds, E.E., Stewart, R.W., Bond, M. (1989) Endothelin is a positive inotropic agent in human and rat heart in vitro, Biochemical and Biophysical Research Communications, 159, 14-19

Moreland, S., McMullen, D.M., Delaney, C.L., Lee, V.G., Hunt, J.T. (1992) Venous smooth muscle contains vasoconstrictor ET(B)-like receptors, Biochemical and Biophysical Research Communications, 184, 100-106

Morii, S., Ngai, A.C., Winn, H.R. (1984) Attenuation of hypoxic hyperaemia by theophylline: The role of adenosine in cerebral blood flow regulation, Surgical Forum, 35, 504-507

Morikawa, E., Ginsberg, M.D., Dietrich, W.D., Duncan, R.C., Kraydieh, S., Globus, M.Y-T. , Busto, R. (1992) The significance of brain temperature in focal

cerebral ischaemia, Histopathological consequences of middle cerebral artery occlusion in the rat, Journal of Cerebral Blood Flow and Metabolism, 12, 380-389.

Morita, T., Kurihara, H., Maemura, K., Yoshizumi, M., Yazaki, Y. (1993) Disruption of cytoskeletal structures mediates shear stress- induced endothelin-1 gene expression in cultured porcine aortic endothelial cells, Journal of Clinical Investigation, 92, 1706-1712

Moskowitz, M.A., Brody M., Liu-Chen, L-Y. (1983) In vitro release of immunoreactive substance P from putative afferent nerve endings in bovine pia arachnoid, Neuroscience, 9, 809-814

Moskowitz, M.A., Macfarlane, R., Tasdemiroglu, E., Wei, E.P., Kontos, H.A. (1990) Neurogenic control of the cerebral circulation during global ischaemia, Stroke, 21 (Suppl. III), III168-III171.

Muir, K.W., Lees, K.R. (1994) Clinical experience with excitatory amino acid antagonist drugs, Stroke, 26, 503-513

Muramatsu, K., Tomoike, H., Ohara, Y., Egashra, S., Nakamura, M. (1991) Effects of endothelin-1 on epicardial coronary tone, coronary blood flow, ECG ST change and regional wall motion in anaesthetised dogs, Heart Vessels, 6, 191-196

Murata, S., Matsumura, Y, Takada, K, Asai, Y, Takaoka, M, Morimoto, S. (1995) Role of transforming growth factor ß1 on platelet induced enhancement of endothelin-1 production in cultured vascular endothelial cells, Journal of Pharmacology and Experimental Therapeutics, 274, 1524-1530

Murphy, S., Simmons, M.L., Agullo, L., Garcia, A., Feinstein, D.L., Galea, E., Reis, D.J., Minc-Golomb, D., Schwartz, J.P. (1993) Synthesis of nitric oxide in CNS glial cells, Trends in Neurosciences, 16, 323-328

Murray, M.A., Faraci, F.M., Heistad, D.D. (1992) Effect of protein kinase C inhibitors on endothelin- and vasopressin- induced constriction of the rat basilar artery, American Journal of Physiology, 263, H1643-H1649

Nambi, P., Elshourbagy, N.A., Wu, H-L., Pullen, M., Ohlstein, E.H., Brooks, D.P., Lago, M.A., Elliot, J.D., Gleason, J.G., Ruffolo, R.R. (1994) Non peptide endothelin receptor antagonists. 1 Effects on binding and signal transduction on human ETA and

ETB receptors, Journal of Pharmacology and Experimental Therapeutics, 271, 755-761

Nambi, P., Pullen, M., Feuerstein, G.Z. (1990) Identification of endothelin receptors in various regions of the rat brain , Neuropeptides, 16, 195-199

Nambi P., Pullen, M., Jugus, M., Gellai, M. (1993) Rat kidney endothelin receptors in ischaemia induced acute renal failure, Journal of Pharmacology and Experimental Therapeutics, 264, 345-348

Namiki, A., Hirata, Y., Ishikawa, M., Moroi, M., Aikawa, J., Machii, K. (1992) Endothelin-1 and endothelin-3 induced vasorelaxation via common generation of endothelium derived nitric oxide, Life Sciences, 50, 677-682

Naritomi, H., Sasaki, M., Kanashiro, M., Kitani, M., Sawada, T. (1988) Flow thresholds for cerebral energy disturbance and Na+ pump failure as studied by in vivo 31P and 32Na nuclear magnetic resonance spectroscopy, Journal of Cerebral Blood Flow and Metabolism, 8, 16-23

Nathan, C. (1992) Nitric oxide as secretory product of mammalian cells FASEB Journal 6, 3051-3064

Navari, R.M., Wei, E.P., Kontos, H.P., Patterson, J.L. (1978) Comparison of the open skull and cranial window preparations in the study of the cerebral microcirculation, Microvascular Research, 16, 304-315

Nayler, W.G., Ou, R.G., Gu, X.H., Casley, D.H. (1992) Effect of amlodipine pretreatment on ischaemia reperfusion induced increase in cardiac endothelin-1 binding site density, Journal of Cardiovascular Pharmacology, 20, 416-420

Nedergaard, M. , Diemer, N.H. (1987) Focal ischaemia in the rat, with special reference to the influence of plasma glucose concentration, Acta Neuropathologica (Berl.), 73, 131-137.

Nellgård, B., Gustafson, I. , Wieloch, T. (1991) Lack of protection by the N-methyl-D-aspartate receptor blocker dizocilpine (MK-801) following transient severe cerebral ischaemia in the rat, Anaesthesiology, 75, 279-287.

Neubauer, S., Zimmermann, S., Hirsh, A., Pulzer, F., Tian, R., Bauer, B., Ertl, G. (1991) Effects of endothelin-1 in the isolated heart in ischaemia/reperfusion and

hypoxia/reoxygenation injury, Journal of Molecular and Cellular Cardiology, 23, 1397-1409

Ng, T., Graham, D.I., Adams, J.H., Ford, I. (1989) Changes in the hippocampus and the cerebellum resulting from hypoxic insults: frequency and distribution, Acta Neuropathologica, 78, 438-443

Nicholson, C., Brugggencate, G.T., Steinberg, R. , Stockle, H. (1977) Calcium modulation in brain extracellular microenvironment demonstrated with ion-selective micropipette, Proceedings of the National Academy of Sciences, U.S.A., 74, 1287-1290.

Nicoletti, F., Iadarola, M.J., Wroblewski, J.T., Costa, E. (1986) Excitatory amino acid recognition sites coupled with inositol phospholipid metabolism: Developmental changes and interaction with alpha1-adrenoceptors, Proceedings of the National Academy of Sciences of the United States of America, 83, 1931-1935

Nikolov, R., Rami, A., Krieglstein, J. (1993) Endothelin-1 exacerbates focal cerebral ischaemia without exerting neurotoxic action in vitro, European Journal of Pharmacology (Environmental and Toxicological Pharmacology Sect), 248, 205.

Nirei, H., Hamada, K., Shoubo, M., Sogabe, K., Notsu, Y., Ono, T. (1993) An endothelin (ETA) receptor antagonist FR139317 ameliorates cerebral vasospasm in dogs, Life Sciences, 52, 1869-1874

Nishikibe, M., Tsuchida, S., Okada, M., Fukuoda, T., Shimamoto, K., Yano, M., Ishikawa, K., Ikemoto, F. (1993) Antihypertensive effect of a newly synthesised endothelin antagonist BQ-123 in a genetic hypertensive model, Life Sciences, 52, 717-724

Nishimura, H., Rosenblum, W.I., Nelson, G.H., Boynton, S. (1991) Agents that modify EDRF formation alter antiplatelet properties of brain arteriolar endothelium in vivo, American Journal of Physiology, 261, H15-H21

Niwa, M., T. Kawaguichi, T. Himeno, M. Fujimoto, M. Kurihama, K. Yamashita, Y. Katsaoka, K. Shigematsu and K. Taniyama, 1992, Specific binding sites for 125I-endothelin-1 in the porcine and human spinal cord, European Journal of Pharmacology (Mol. Pharmacology. Sect.) 225, 281.

Nozaki, K., Moskowitz, M.A., Maynard, K.I., Koketsu, N., Dawson, T.M., Bredt, D.S., Snyder, S.H. (1993) Possible origin and distribution of immunoreactive nitric oxide synthase-containing nerve fibres in cerebral arteries, Journal of Cerebral Blood Flow and Metabolism, 13, 70-79

O'Brien, M.D. , Waltz, A.G. (1973) Transorbital approach for occluding the middle cerebral artery without craniectomy, Stroke, 4, 201-206.

Ogura, K., Takayasu, M., Dacey, R.G. (1991) Differential-effects of intraluminal and extraluminal endothelin on cerebral arterioles, American Journal Of Physiology, 261

Ohlstein, E.H. , Storer, B. (1992) Endothelin may be the mediator of oxyhaemoglobin- induced cerebral vasospasm. Circulation 86, 288-290

Ohlstein, E.H., Nambi, P., Douglas, S.A., Edwards, R.M., Gellai, M., Lago, A., Leber, J.D., Cousins, R.D., Gao, A., Frazee, J.S., Peishoff, C.E., Bean, J.W., Eggleston, D.S., Elshourbagy, N.A., Kumar, C., Lee, J.A., Yue, T-L., Louden, C., Brooks, D.P., Weinstock, J., Feuerstein, G., Poste, G., Ruffolo, R.R., Gleason, J.G., Elliot, J.D. (1994) SB209670, a rationally designed potent non peptide endothelin receptor antagonist, Proceedings of the National Academy of Sciences USA, 91, 8052-8056

Ohlstein, E.H., Storer, B.L., Butcher, J.A., Debouck, C., Feuerstein, G. (1991) Platelets stimulate expression of endothelin mRNA and endothelin biosynthesis in cultured endothelial cells, Circulation Research, 69, 832-841

Ohno, K., Pettigrew, K.D. , Rapoport, S.I. (1978) Lower limits of cerebrovascular permeability to non electrolytes in the conscious rat, American Journal of Physiology, 235, H299-H307.

Okabe, H., Chijiiwa, Y., Nakamura, K., Yoshinaga, M., Akiho, H., Harada, N., Nawata, H. (1995) Two endothelin receptors (ET(A) and ET(B)) expressed on circular smooth muscle cells of guinea pig cecum, Gastroenterology, 108, 51-57

Opgenorth, T.J., Wu-Wong, J.R., Shiosaki, K. (1992) Endothelin converting enzymes, FASEB Journal, 6, 2653-2659

Osborne, K.A., Shigeno, T., Balarsky, A.-M., Ford, I., McCulloch, J., Teasdale, G.M. , Graham, D.I. (1987) Quantitative assessment of early brain damage in a rat model of focal cerebral ischaemia, Journal of Neurology, Neurosurgery and Psychiatry, 50, 402-410.

Ota, S., Hirata, Y., Sugimoto, T., Kohmoto, O., Hata, Y., Yoshiura, K., Nakada, R., Terano, A. (1991) Endothelin-1 secretion from cultured rabbit gastric epithelial cells, Journal of Cardiovascular Pharmacology, 17 (Suppl. 7), S406-S407

Ottosson, A., Hill, S.J., Edvinsson, L. (1990) Histamine receptors in brain vessels of guinea-pig: In-vitro pharmacology and ligand binding, Acta Physiologica Scandinavica, 140, 135-141

Ottosson, A., Jansen, I., Edvinsson, L. (1988) Characterisation of histamine receptors in isolated human cerebral arteries, British Journal of Pharmacology, 94, 901-907

Ottosson, A., Jansen, I., Edvinsson, L. (1989) Pharmacological characterisation of histamine receptors in the human temporal artery, British Journal of Clinical Pharmacology, 27, 139-145

Overgaard., K. (1994) Thrombolytic therapy in experimental embolic stroke Cerebrovascular and Brain Metabolism Reviews, 6, 257-286

Ozyurt, E., Graham, D.I., Woodruff, G.N. , McCulloch, J. (1988) Protective effect of the glutamate antagonist, MK-801 in focal cerebral ischaemia in the cat, Journal of Cerebral Blood Flow and Metabolism, 8, 138-143.

Panek, R.L., Major, T.C., Hingorani, G.P., Doherty, A.M., Taylor, D.G., Rapundalo, S.T. (1992) Endothelin and structurally related analogs distinguish between endothelin receptor subtypes, Biochemical and Biophysical Research Communications, 183, 566-571

Papadopoulos, S.M., Gilber, L.L., Webb, R.C., D'Amato, C.J. (1990) Characterisation of contractile responses to endothelin in human cerebral arteries: implications for cerebral vasospasm, Neurosurgery, 26, 810-815

Park, C.K., Nehls, D.G., Graham, D.I., Teasdale, G.M. , McCulloch, J. (1988a) Focal cerebral ischaemia in the cat, Treatment with the glutamate antagonist MK-801 after induction of ischemia, Journal of Cerebral Blood Flow and Metabolism, 8, 757-762.

Park, C.K., Nehls, D.G., Graham, D.I., Teasdale, G.M. , McCulloch, J. (1988b) The glutamate antagonist MK-801 reduces focal ischemic brain damage in the rat, Annals of Neurology, 24, 543-551.

Park, C.K., Nehls, D.G., Teasdale, G.M. , McCulloch, J. (1989) Effect of the NMDA antagonist MK-801 on local cerebral blood flow in focal cerebral ischaemia in the rat, Journal of Cerebral Blood Flow, 9, 617-622.

Park, T.S., Van Wylen, D.G.L., Rubio R., Berne R.M. (1987) Increased brain interstitial fluid adenosine concentration during hypoxia in newborn piglet, Journal of Cerebral Blood Flow and Metabolism, 7, 178-183

Paschen, W., Djuricic, B., Mies, G. (1987) Lactate and pH in the brain: Association and dissociation in different pathophysiological states, Journal of Neurochemistry, 48, 154-159

Patel JB , Wilson C Endothelin BQ123 in the SH rat focal ischaemia model (abstract no. P173) 4th International conference on Endothelin, (1995) 23-26 April; London U.K.

Patel, T.R., Galbraith, S., Graham, D.I., Hallak, H., Doherty, A.M., McCulloch, J. (1996) Endothelin (ETA) receptor antagonist increases cerebral perfusion and reduces ischaemic damage in feline focal cerebral ischaemia, Journal of Cerebral Blood Flow and Metabolism, in press

Patlak, C.S., Blasberg, R.G., Fenstermacher, J.D. (1984) An evaluation of errors in the determination of blood flow by the indicator and tissue equilibration (Kety) methods, Journal of Cerebral Blood Flow and Metabolism, 4, 47-60

Paul, K.S., Whalley, E.T., Forster, B.C. (1982) Prostacyclin and cerebral vessel relaxation, Journal of Neurosurgery, 57, 334-340

Paulson, O.B., Strandgaard, S. , Edvinsson, L. (1990) Cerebral autoregulation, Cerebrovascular and Brain Metabolism Reveiws, 2, 161-192.

Paxinos, G. , Watson, C. (1986) The Rat Brain in Stereotaxic Co-ordinates, 2nd edition, Academic Press, Sydney.

Perico, N., Cornejo, R.P., Benigni, ., Malachini, B., Ladny, J.R., Remuzzi, G. (1991) Endothelin induces diuresis and natriuresis in the rat by acting on proximal tubular cells through a mechanism mediated by lipoxygenase products, Journal of the American Society of Nephrology, 2, 57-69

Petito, C.K., Feldmann, E., Pulsinelli, W.A. , Plum, F. (1987) Delayed hippocampal damage in humans following cardiorespiratory arrest, Neurology, 37, 1281-1286.

Phillis, J.W. (1994) A radical view of cerebral ischaemic injury, Progress in Neurobiology, 42, 441-448

Phillis, J.W., Simpson, R.E., Walter, G.A. (1990) The effect of hyperglycemia on extracellular levels of adenosine in the hypoxic rat cerebral cortex, Brain Research, 524, 336-338

Phillis, J.W., Walter, G.A., O'Regan, M.H., Stair, R.E. (1987) Increases in cerebral cortical perfusate adenosine and inosine concentrations during hypoxia and ischemia, Journal of Cerebral Blood Flow and Metabolism, 7, 679-686

Pierre, L.N., Davenport, A.P. (1995) Autoradiography study of endothelin receptors in human cerebral arteries, Journal of Cardiovascular Pharmacology, 26 (Suppl. 3), S326-SS328

Plane, F., Garland, C.J. (1993) Differential effects of acetylcholine, nitric oxide and levocromakalim on smooth muscle potential and tone in the rabbit basilar artery, British Journal of Pharamcology, 110, 651-656

Pollock, D.M., Opgenorth, T.J. (1993) Evidence for endothelin-induced renal vasoconstriction independent of ET(A) receptor activation, American Journal of Physiology, 264, R222-R226

Pulsinelli WA, Levy DE, Duffy TE (1982b) Regional cerebral blood flow and glucose metabolism following transient ischaemia. Annals of Neurology 11,499-509.

Pulsinelli, W.A. , Brierley, J.B. (1979) A new model of bilateral hemispheric ischemia in the unanaesthetized rat, Stroke, 10, 267-272.

Pulsinelli, W.A. , Duffy, T.E. (1983) Regional energy balance in rat brain after transient forebrain ischemia, Journal of Neurochemistry, 40, 1500-1503.

Pulsinelli, W.A., Brierley, J.B. , Plum, F. (1982a) Temporal profile of neuronal damage in a model of transient forebrain ischemia, Annals of Neurology, 11, 491-498.

Qizilbash, N., Dufy, S.W., Warlow, C., Mann J. (1992) Lipids are risk factors for iscahemic stroke, overview and review, Cerebrovascular Diseases, 2, 127-136

Raper, A.J., Kontos, H.A, Patterson, J.L. (1971) Response of pial precapillary vessels to changes in arterial carbon dioxide concentration, Circulation Research, 28, 518-523

Rapoport, R.M., Murad, F. (1983) Agonist-induced endothelium dependent relaxation in rat thoracic aorta may be mediated through cyclic GMP Circulation Research 52, 352-357

Rand, V.E., Garland, C.J. (1992) Endothelium-dependent relaxation to acetylcholine in the rabbit basilar artery: importance of membrane hyperpolarisation, British Journal of Pharmacology, 106, 143-150

Ray, S.G., McMurray, J.J., Morton, J.J., Dargie, H.J. (1992) Circulating endothelin in acute ischemic syndromes, British Heart Journal, 67, 383-386

Reese, T.S., Karnovsky, M..J (1967) Fine structural localisation of a blood-brain barrier to exogenous peroxidase, Journal of Cell Biology, 34, 207-217

Regoli, D. (1984) Neurohumoral regulation of pre-capillary vessels, the kallikrein-kinin system, Journal of Cardiovascular Pharmacology, 6 (Suppl. 2), S401-S412

Regoli, D., Barabe, J. (1980) Pharmacology of bradykinin and related kinins, Pharmacology Reviews, 32, 1-46

Regoli, D., Rouissi, N., D'Orleans-Juste, P. (1993) Characterisation of neurokinin receptors in the circulation In, Vascular innervation and receptor mechanisms, new perspectives ed. Edvinsson L, Uddman R Academic press, London pp 281-297

Reinoso-Suarez, F. (1961) Topograpischer Hirnatlas der Katze, fur experimental-physiologische Untersuchungen. Darmstadt, E. Merck.

Reivich, M., Jehle, J., Sokoloff, L., Kety, S.S. (1969) Measurement of regional cerebral blood flow with antipyrine in awake cats, Journal of Applied Physiology, 27, 296-300

Resink, T.J., Hahn, A.W.A., Scott-Burden, T. (1990) Inducible endothelin mRNA expression an peptide secretion in culture in human vascular smooth muscle cells, Biochemical Biophysical Research Communications, 168, 1303-1310

Reynolds, E.E., Keiser, J.A., Haleen, S.J., Walker, D.M., Olszewski, B., Schroeder, R.L., Taylor, D.G., Hwang, O., Welch, K.M., Flynn, M.A., Thompson, D.M., Edmunds, J.J., Berryman, K.A., Plummer, M., Cheng, X.M., Patt, W.C., Doherty, A.M. (1995) Pharmacological characterisation of PD156707, an orally active ET(A) receptor antagonist, Journal of Pharmacology and Experimental Therapeutics, 273, 1410-1417

Ridenour, T.R., Warner, D.S., Todd, M.M. , McAllister, A.C. (1992) Mild hypothermia reduces infarct size resulting from temporary but not permanent focal ischemia in rats, Stroke, 23, 733-738.

Riezebos, J., Watts, I.S., Vallance, P.J.T. (1994) Endothelin receptors mediating functional responses in human small arteries and veins, British Journal of Pharmacology, 111, 609-615

Robinson, M.J., Macrae, I.M., Todd, M., Reid, J.L., McCulloch, J. (1990) Reduction of local cerebral blood flow to pathological levels by endothelin-1 applied to the middle cerebral artery in the rat, Neuroscience Letters, 118, 269-272

Robinson, M.J., McCulloch, J. (1990) Contractile responses to endothelin in feline cortical vessels in situ, Journal of Cerebral Blood Flow and Metabolism, 10, 265-289.

Robinson, R.G., Shoemaker, W.J., Schlumpf, M., Valk, T. , Bloom, F.E. (1975) Experimental early infarction in rat brain, effect on catecholamines and behaviour, Nature, 255, 332-334.

Rorstad, O., Shirahase, H., Huang, M. (1993) Pharmacology of acetylcholine and vasoactive intestinal peptide in perivascular nerves In, Vascular innervation and receptor mechanisms, new perspectives ed. Edvinsson L, Uddman R Academic press, London pp 221-243

Rosenblum, W.I., Nelson, G.H., Povlishock, J.T. (1987) Laser induced endothelial damage inhibits endothelium dependent relaxation in the cerebral microcirculation of the mouse, Circulation Research, 60, 169-176

Rosenblum, W.I., Nishimura, H., Ellis, E.F., Nelson, G.H. (1992) The endothelium dependent effects of thimerosal on mouse pial arterioles in vivo, evidence for control of microvascular events by EDRF as well as prostaglandins, Journal of Cerebral Blood Flow and Metabolism, 12, 703-706

Rosenblum, W.I., Kontos, H.A. (1974) The importance and relevance of studies of the pial microcirculation, Stroke, 5, 425-428

Rosenfeld, M.G., Mermod, J-J., Amara, S.G., Swanson, L.W., Sawchenko, P.E., Rivier, J., Vale, J.J. (1983) Production of novel neuropeptide encoded by the calcitonin gene via tissue specific RNA processing, Nature, 304, 129-135

Rostene, W.H. (1984) Neurobiological and neuroendocrine functions of the vasoactive intestinal peptide (VIP), Progress in Neurobiology, 22, 103-129

Rothwell, N.J., Relton, J.K. (1993) Involvement of interleukin-1 and lipocortin-1 in ischaemic damage, Cerebrovascular and Brain Metabolism Reviews, 5, 178-198

Roux, S.P., Loffler, B-M., Gray, G., Clozel, M. (1995) The role of endothelin in experimental cerebral vasospasm, Neurosurgery. 37, 78-85

Rubanyi, G.M., Polokoff, M.A. (1994) Endothelins, molecular biology, biochemistry, pharmacology , physiology and pathophysiology, Pharmacology Reviews, 46, 325-415 .

Sacco, R.L. (1993) Current epidemiology of stroke In, Current review of cerebrovascular disease eds. Fisher, M., Bogousslavsky, J. Current Medicine Inc., Philadelphia pp3-14

Sacco, R.L., Hansen, W.A., Mohr, J.P. (1991) Hospitalised stroke incidence in blacks and hispanics in northern Manhattan, Stroke, 22, 1491-1496

Saeki, T, Ihara, M., Fukuroda, T., Yamagiwa, M., Yano, M. (1991) (Ala(1,3,11,15))endothelin-1 analogs with ET(B) agonistic activity, Biochemical and Biophysical Research Communications, 179, 286-292

Sagher, O., Jin, Y., Thai, Q.A., Fergus, A., Kassell, N.F., Lee, K.S. (1994) Cerebral microvascular responses to endothelins: The role of ET(A) receptors, Brain Research, 658, 179-184

Sahuquillo-Barris, J., Lamarca-Ciuro, J., Vilatta-Castan, J., Rubio-Garcia, E., Rodriguez-Pazos, M. (1988) Acute subdural haematoma and diffuse axonal injury after severe head trauma, Journal of Neurosurgery, 68, 894-900

Saito, A., Shiba, R. Kimura, S., Yanagisawa, M., Goto, K., Masaki, T. (1989) Vasoconstrictor response of large cerebral arteries of cats to endothelin, an endothelium derived vasoactive peptide, European Journal of Pharmacology, 162, 353-358

Saito, A., Shiba, R., Yanagisawa, M., Masaki, T., Kimura, S., Yamada, K., Mima, T., Shigeno, T., Goto, K. (1991) Endothelins, vasoconstrictor effects and localisation in canine cerebral arteries, British Journal of Pharmacology, 103, 1129-1135

Saito, K., Moskowitz, M.A. (1989) Contributions from upper cervical dorsal roots and trigeminal ganglia to the feline circle of Willis, Stroke, 20, 524-526

Saito, T., Fushimi, E., Abe, T., Kimura, Y., Takahashi. K., Kudo, Y., Nakagomi, A., Miura, M. (1992) Augmented contractile response to endothelin and blunted endothelium dependent relaxation in post-ischaemic reperfused coronary arteries, Japanese Circulation Journal, 56, 657-664

Saito, Y., Kazuwa, N., Shirakami, G., Mukoyama, M., Arai, H., Hosoda, K., Suga, S., Ogawa, Y., Imura, H. (1991) Endothelins in patients with chronic renal failure, Journal of Cardiovascular Pharmacology, 17 (Suppl. 7), S437-S439

Sakurada, O., Kennedy, C., Jehle, J.., Brown, J.D., Carbin, G.L., Sokoloff, L. (1978) Measurement of local cerebral blood flow with iodo[^{14}C]antipyrine, American Journal of Physiology, 234, H59-H66.

Sakurai, T., Yanagisawa, M., Takuwa, Y., Miyazaki, H., Kimura, S., Goto, K., Masaki, T. (1990) Cloning of cDNA encoding a non isopeptide selective subtype of the endothelin receptor, Nature, 348, 732-735.

Salom, J.B., Torregrosa, G., Alborch, E. (1995) Endothelins and the cerebral circulation, Cerebrovascular and Brain Metabolism Reviews, 7, 131-152

Salom, J., Torregrossa, G., Barbera, M., Jover, T., Alborch, E. (1993) Endothelin receptors mediating contraction in goat cerebral arteries, British Journal of Pharmacology, 109, 826-830

Salom, J., Torregrossa, G., Miranda, F., Alabadi, J., Albroch, E, (1992) Comparison of the contractile effects of endothelin-1 and sarafotoxin 6b in goat isolated cerebral arteries, British Journal of Pharmacology, 106, 95-100

Salom, J.B., Torregrosa, G., Miranda, F.J., Alabadi, J.A., Alborch, E. (1991) Effects of endothelin-1 on the cerebrovascular bed of the goat, European Journal of Pharmacology, 192, 39-45

Salvati, P., Chierchia, S., Dho, L., Ferrario, R.G., Parenti, P., Vicedomini, G., Patrono, C. (1991) Proarrhythmic activity of intracoronary endothelin in dogs: relation to the site of administration and to changes in regional flow, Journal of Cardiovascular Pharmacology, 17, 1007-1014

Samson, W.K., Skala, K.D., Alexander, B.D., Huang, H.L. (1990) Pituitary site of action of endothelin:selective inhibition of prolactin release in vitro, Biochemicalical Biophysical Research Communications, 169, 737-743

Satoh, M., Shimura, S., Ishihara, H., Nagaki, M., Sasaki, H., Takishima, T. (1992) Endothelin-1 stimulates chloride secretion across canine tracheal epithelium, Respiration, 59, 145-150

Schilling, L., Feger, G.I., Ehrenreich, H., Wahl, M. (1995) Endothelin-3 induced relaxation of isolated rat basilar artery is mediated by an endothelial ETB type endothelin receptor, Journal of Cerebral Blood Flow and Metabolism, 15, 699-705

Schmidt, M., Kroger, B., Jacob, E., Seulberger, H., Subkowski, T., Otter, R., Meyer, T., Schmalzing, G., Hillen, H. (1995) Molecular characterisation of human and bovine endothelin converting enzyme (ECE-1), FEBS Letters, 356, 238-243

Schmidt-Kastner, R., Freund, T.F. (1991) Selective vulnerability of the hippocampus in brain ischemia, Neuroscience, 40, 599-636

Schoeffter, P., Randriantsoa, A., Jost, B., Bruttel, K. (1993) Comparative effects of the two endothelin ET(A) receptor antagonists, BQ-123 and FR139317, on endothelin-1-induced contraction in guinea-pig iliac artery, European Journal of Pharmacology, 241, 165-169

Seelig, J.M., Becker, D.P., Miller, J.D., Greenberg, R.P., Ward, J.D., Choi, S.C. (1981) Traumatic acute subdural haematoma. Major mortality reduction in comatose patients treated within four hours, New England Journal of Medicine, 304, 1511-1518

Seifert, V., Loffler, B-M., Zimmermann, M., Roux, S., Stolke, D. (1995) Endothelin concentrations in patients with aneurysmal subarachnoid haemorrhage, Journal of Neurosurgery, 82, 55-62

Seo, B.G., Oemar, B.S., Siebenmann, R., von Segesser, L., Luscher, T.F. (1994) Both ETA and ETB receptors mediate contraction to endothelin-1 in human blood vessels, Circulation, 89, 1203-1208.

Sercombe, R., Verrecchia, C., Philipson, V. (1986) Histamine-induced constriction and dilatation of rabbit middle cerebral arteries in vitro: Role of the endothelium, Blood Vessels, 23, 137-153

Seylaz, J., Hara, H., Pinard, E., Mraovitch, S., MacKenzi, E.T., Edvinsson, L. (1988) Effect of stimulation of the sphenopalatine ganglion on cortical blood flow in the rat, Journal of Cerebral Blood Flow and Metabolism, 8, 875-878

Shaper, A.G., Philips, A.N., Pocock, S.J., Walker, M., Macfarlane, P.W. (1991) Risk factors for stroke in middle aged British men, British Medical Journal, 302, 1111-1115

Sharkey, J., Ritchie, I.M., Kelly, P.A.T. (1993) Perivascular microapplication of endothelin-1, A new model of focal cerebral ischaemia in the rat, Journal of Cerebral Blood Flow and Metabolism, 13, 865-871

Sharma, M., Hakim, A.M (1993) Neuroscience of cerebral ischaemia, In Current Review of cerebrovascular disease, eds. Fisher, M., Bougousslavsky, J., Current Medicine, Philadelphia pp. 15-22

Shetty, S.S., Okada, T., Webb, R.L., Del Grande, D., Lappe, R.W. (1993) Functionally distinct endothelin ETB receptor in vascular endothelium and smooth muscle, Biochemical and Biophysical Research Communications, 191, 459-464

Shigeno, T., Clozel, M., Sakai, S. (1995) The effect of bosentan, a new potent endothelin receptor antagonist, on the pathogenesis of cerebral vasospasm, Neurosurgery, 37, 87-90

Shimada, N., Graf, R., Rosner, G. , Heiss, W.-D. (1990) Differences in ischemia-induced accumulation of amino acids in the cat cortex, Stroke, 21, 1445-1451.

Shimada, N., Graf, R., Rosner, G., Wakayama, A., George, C.P. , Heiss, W.-D. (1989) Ischemic flow threshold for extracellular glutamate increase in cat cortex, Journal of Cerebral Blood Flow and Metabolism, 9, 603-606.

Shimizu, K., Miyazaki, M., Toda, N. (1979) Effects of vasodilating agents on isolated dog cerebral and peripheral arteries, Japanese Journal of Pharmacology, 29, 151

Shinmi, O., Kimura, S., Sawamura, T., Sugita, Y., Yoshizawa, T., Uchiyama, Y., Yanagisawa, M., Goto, K., Masaki, T., Kanazawa, I. (1989a) Endothelin-3 is a novel neuropeptide: isolated and sequence determination of endothelin-1 and endothelin-3 in porcine brain, Biochemical and Biophysical Research Communications, 164, 587-593

Shinmi, O., Kimura, S., Yoshizawa, T., Sawamura, T., Uchiyama, Y., Sugita, Y., Kanazawa, I., Yanagisawa, M., Goto, K., Masaki, T. (1989b) Presence of endothelin-1 in porcine spinal cord: isolaton and sequence determination, Biochemical and Biophysical Research Communications, 162, 340-346

Shirakami, G., Magaribuchi, T., Shingu, K., Saito, Y., Nakao, K., Mori, K. (1994) Changes of endothelin concentration in cerebrospinal fluid and plasma of patients with aneurysmal subarachnoid haemorrhage, Acta Anaesthesiologica Scandinavica, 38, 457-461

Shirakami, G., Nakao, K., Saito, Y., Magaribuchi, T., Jongasaki, M., Mukoyama, M., Arai, H., Hosoda, K., Suga, S., Ogawa, Y. (1991) Acute pulmonary alveolar hypoxia increases lung and plasma endothelin-1 levels in conscious rats, Life Sciences, 48, 969-976

Siesjö, B.K. (1981) Cell damage in the brain, a speculative synthesis, Journal of Cerebral Blood Flow and Metabolism, 1, 155-185.

Siesjö, B.K. (1988a) Acidosis and ischemic brain damage, Neurochemical Pathology, 9, 31-88.

Siesjö, B.K. (1988b) Historical overview. Calcium, ischemia, and death of brain cells, Annals of the New York Academy of Sciences, 522, 638-661.

Siesjö, B.K. (1992a) Pathophysiology and treatment of focal cerebral ischemia, Part I, Pathophysiology, Journal of Neurosurgery, 77, 169-184.

Siesjö, B.K. (1992b) Pathophysiology and treatment of focal cerebral ischemia, Part II, Mechanisms of damage and treatment, Journal of Neurosurgery, 77, 337-354.

Siesjö, B.K. , Bengtsson, F. (1989) Calcium fluxes, calcium antagonists, and calcium-related pathology in brain ischemia, hypoglycemia, and spreading depression, a unifying hypothesis, Journal of Cerebral Blood Flow and Metabolism, 9, 127-140.

Simonson, M.S., Jones, J.M., Dunn, M.J. (1992) Differential regulation of fos and jun gene and AP1 cis element activity by endothelin isopeptides. possible implication for mitogenic signalling by endothelins, Journal of Biological Chemistry, 267, 8643-8649

Siney, L., Lewis, M.J. (1994) Nitric oxide modulates endothelin release from porcine cultured endocardial endothelium, Endothelium: Journal of Endothelial Cell Research, 2, 169-175

Siren, A.L., Feuerstein, G.Z. (1989) Haemodynamic effects of endothelin after systemic and central nervous system administration in the conscious rat, Neuropeptides, 14, 231-236

Skarby TVC, Andersson KE, Edvinsson L (1983) Pharmacological characterisation of postjunctional α-adrenoceptors in isolated feline cerebral and peripheral arteries Acta Physiologica Scandanavica 117, 63-67

Skofitsch, G., Jacoboitz, D.M. (1985) Autoradiographic distribution of ^{125}I calcitonin gene-related peptide binding sites in the rat central nervous system, Peptides, 4, 975-986

Sladeczek, F., Recasens, M., Bockaert, .J (1988) A new mechanism for glutamate receptor action: Phosphoinositide hydrolysis, Trends in Neurosciences, 11, 545-549

Smith, M.-L., Auer, R.N. , Siesjö, B.K. (1984) The density and distribution of ischemic brain injury in the rat following 2-10 min of forebrain ischemia, Acta Neuropathologica, 64, 319-332.

Sogabe K, Nirei H, Shoubo M, Nomoto A, Ao S, Notsu Y, Ono T 1993 Pharmacological profile of FR139317, a novel potent endothelin ETA receptor antagonist, Journal of Pharmacology and Experimental Therapeutics. 264,1040-1046

Spatz, M., Stanimirovic, D., Strasser, A., McCarron, R. M. (1995) Nitro-L-arginine augments endothelin-1 content of cerebrospinal fluid induced by cerebral ischaemia, Brain Research, 684, 99-104

Speth, R.C., Harik, S.I. (1985) Angiotensin II receptor binding sites in brain microvessels, Proceedings of the National Academy of Sciences U.S.A., 82, 6340-6343

Stanismirovic, D., McCarron, R., Bertrand, N., Spatz, M. (1993) Endothelins release ^{51}Cr from cultured human cerebromicrovascular endothelium, Biochemical and Biophysical Research Communications, 191, 1-8

Steen, P.A., Newberg, L.A., Milde, J.H., Michenfelder, J.D. (1984) Cerebral blood flow and neurologic outcome when nimodipine is given after complete cerebral ischaemia in the dog, Journal of Cerebral Blood Flow and Metabolism, 4, 82-87.

Steen, S., Skarby, T.V.C., Norgren, L., Andersson, K.E. (1984) Pharmacological characterization of postjunctional alpha-adrenoceptors in isolated human omental arteries and veins, Acta Physiologica Scandinavica, 120, 109-116

Stewart, D.J., Levy, R.D., Cernacek, P., Langleben, D. (1991) Increased plasma endothelin-1 in pulmonary hypertension: marker or mediator of disease?, Annals of Internal Medicine, 114, 464-469

Stone, J.L., Rifai, M.H.S., Sugar, O., Lang, R.G.R., Oldershaw, J.B., Moody, R.A. (1983) Subdural haematoma I. Acute subdural haematoma progress in definition, clinical pathology and therapy, Surgical Neurology, 19, 216-231

Stosseck, K. (1970) Hydrogen exchange through the pial vessel wall and its meaning for the determination of local cerebral blood flow, Pflugers Archives, 320, 111-119

Stromberg, D.D., Fox, J.R. (1972) Pressures in pial arterial microcirculation of the cat during changes in systemic arterial blood pressure, Circulation Research, 31, 229-239

Strong, A.J., Tomlinson, B.E., Venables, G.S., Gibson, G., Hardy, J.A. (1983a) The cortical ischemic penumbra associated with occlusion of the middle cerebral-artery in the cat Studies of histopathology, water-content, and in vitro neurotransmitter uptake, Journal Of Cerebral Blood Flow And Metabolism , 3 , 97-108

Strong, A.J., Venables, G.S. , Gibson, G. (1983b) The cortical ischaemic penumbra associated with occlusion of the middle cerebral artery in the cat, 1. Topography of changes in blood flow, potassium ion activity and EEG, Journal of Cerebral Blood Flow and Metabolism, 3, 86-96.

Stys, P.K., Waxman, S.G., Ransom, B.R. (1991) Na+-Ca2+ exchanger mediates Ca2+ influx during anoxia in mammalian central nervous system white matter, Annals of Neurology, 30, 375-380

Sudwarjo, S., Hori, M., Takai, M., Urade, Y., Okada T, Karaki, H. (1993) A novel subtype of endothelin B receptor mediating contraction in swine pulmonary vein, Life.of Sciences, 53, 431-437

Sumner, M., Cannon, T., Mundi , J., White, D., Watts, I. (1992) Endothelin ET_A and ET_B receptor mediate vascular smooth muscle contraction, British Journal of Pharmacology, 107,858-860

Sutherland, G., Lesiuk, H., Bose, R. , Sima, A. (1988) Effect of mannitol, nimodipine and indomethacin singly or in combination on cerebral ischemia in rats, Stroke, 19, 571-578.

Suzdak, P.D., Sheardown, M.J. (1993) Effect of the non NMDA receptor antagonist 2, 3 - Dihydro - 6 - Nitro - 7 -Sulfamoyl benzo (f) quinoxaline on local cerebral glucose uptake in the limbic forebrain, Journal of Neurochemistry, 61, 1577-1580

Suzuki, N., Hardebo, J.E., Owman, C. (1989) Origin and pathways of cerebrovascular nerves storing substance P and calcitonin gene-related peptide in the rat, Neuroscience, 31, 427-438

Suzuki, R., Masaoka, H., Hirata, Y., Marumo, F., Isotani, E. , Hirakawa, K. (1992) The role of endothelin-1 in the origin of cerebral vasospasm in patients with aneurysmal subarachnoid hemorrhage. Journal of Neurosurgery 77, 96-100

Symon, L (1970) Regional vascular reactivity in the middle cerebral arterial distribution: an experimental study in baboons, Journal of Neurosurgery, 33, 532-541

Symon, L., Branston, N.M. , Strong, A.J. (1976) Autoregulation in acute focal ischemia, Stroke, 7, 547-554.

Tabuchi, Y., Nakamura, M., Rakugi, H., Nagano, M., Ogihara, T. (1989) Endothelin-1 inhibits presynaptic adrenergic neurotransmission in rat mesenteric artery, Biochemical and Biophysical Research Communications, 161, 803-808

Taga, K., Fukuda,S., Nishimura, N., Tsukui, A., Morioka, M., Shimoji, K. (1990) Effects of thiopental, pentobarbital and ketamine on endothelin induced constriction of porcine cerebral arteries, Anaethesiology, 72, 939-941

Takai, M., Umemura, I., Yamasaki, K., Watakabe, T., Fujitani, Y., Oda, K., Urade, Y., Inui, T., Yamamura, T., Okada, T. (1992) A potent and specific agonist, Suc-(Glu9,Ala11,15)-endothelin-1(8- 21), IRL 1620, for the ET(B) receptor, Biochemical and Biophysical Research Communications, 184, 953-959

Takayanagi, R., Kitazumi, K., Takasaki, C., Ohnaka, K., Aimoto, S., Tasaka, K., Ohashi, M., Nawata, H. (1990) Presence of non selective type of endothelin receptor on vascular endothelium and its linkage to vasodilatation, FEBS Letters, 282, 103-106

Takenaka, K., Kishino, J., Arita, H., Okano, Y., Sakai, N., Yamada, H., Nozawa, Y. (1993) Biological activity of the endothelin family in cultured basilar arterial smooth muscle cells, Neurological Research, 15, 29-32

Tamirisa, P., Frishman, W.H., Kumar, A. (1995) Endothelin and endothelin antagonism: roles in cardiovascular health and disease, American Heart Journal, 130, 601-610

Tamura, A., Graham, D.I., McCulloch, J. , Teasdale, G.M. (1981a) Focal cerebral ischaemia in the rat, I. Description of technique and early neuropathological consequences following middle cerebral artery occlusion, Journal of Cerebral Blood Flow and Metabolism, 1, 53-60.

Tamura, A., Graham, D.I., McCulloch, J. , Teasdale, G.M. (1981b) Focal cerebral ischemia in the rat, 2. Regional cerebral blood flow determined by [^{14}C]iodoantipyrine autoradiography following middle cerebral artery occlusion, Journal of Cerebral Blood Flow and Metabolism, 1, 61-69.

Tanaka, Y., Nakayama, K. (1991) Responses of endothelium-intact and -denuded feline and canine cerebral arteries to quick stretch, Asia Pacific Journal of Pharmacology, 6, 159-163

Tanoi, C., Suzuki, Y., Masato, S., Suguita, K., Masuzawa, K., Asano, M. (1991) Mechanism of the enhanced vasoconstrictor responses to endothelin-1 in canine cerebral arteries, Journal of Cerebral Blood Flow Metabolism, 11, 371-379

Tasdemiroglu, E., Macfarlane, R., Wei, E.P., Kontos, H.A., Moskowitz,M.A. (1992) Pial vessel caliber and cerebral blood-flow become dissociated during ischemia-reperfusion in cats, American Journal of Physiology , 263 , 533-536

Tayag, E.C., Katolik, L.I., Lin, R.C.S., Nair, S.N., Jeng, A.Y., Yanagisawa, M., Lehmann, J.C. (1995) Endothelin-1 and endothelin converting enzyme-1 following experimental traumatic brain injury in the rat, Journal of Neurotrauma, 12, 992 (Abstract TB31)

Teasdale, G., Tamura, A., Graham, D., Rabow, L., MacKenzie, E. (1981) Vasoconstriction in focal cerebral ischaemia In, Cerebral Microcirculation and Metabolism eds Cervos-Navarro, J., Fritschka, E., Raven Press, New York, pp 77-81

Teerlink, J.R., Breu, V., Sprecher, U., Clozel, M., Clozel, J.P. (1994) Potent vasoconstriction mediated by endothelin ET(B) receptors in canine coronary arteries, Circulation Research, 74, 105-114

Telemaque, S., Gratton, J.P., Claing, A., Dorleans-Juste, P. (1993) Pharmacological evidence for the specificity of the phosphoramidon-sensitive endothelin-converting enzyme for big endothelin-1, Journal Of Cardiovascular Pharmacology, 22 (Suppl. 8), 85-89

Tence, M., Cordier, J., Glowinski, J., Premont, J. (1992) Endothelin-evoked release of arachidonic acid from mouse astrocytes in primary culture, European Journal of Neuroscience, 4, 993-999

Tippler, B., Herbst, C., Simmet, T. (1994) Evidence for the formation of endothelin by lysed red blood cells from endogenous precursor, European Journal of Pharmacology, 271, 131-139

Toda N, Miyazaki M (1981) Angiotensin induced relaxation on isolated dog renal and cerebral arteries, American Journal of Physiology, 240, H247-H254

Toda, N. (1980) Responses to prostaglandins H2 and I2 of isolated dog cerebral and peripheral arteries, American Journal of Physiology, 7, H111-H117

Toda, N., Okamura, T., Shimizu, I., Tatsuno, Y. (1985) Postmortem functional changes in coronary and cerebral arteries from humans and monkeys, Cardiovascular Research, 19, 707-713

Toda, N., Shimizu, I., Okamura, T., Miyazaki, M. (1986) Age-dependent change in the response of isolated beagle cerebral arteries to vasoactive agents, Journal of Cardiovascular Pharmacology, 8, 681-688

Toda, N. (1980) Responses to prostaglandins H2 and I2 of isolated dog cerebral and peripheral arteries, American Journal of Physiology, 7, H111-H117

Torvik, A. (1984) The pathogenesis of watershed infarcts in the brain, Stroke, 15, 221

Triguero, D., Lopez de Pablo, A.L., Gomez, B., Estrada, C. (1988) Regional differences in cerebrovascular cholinergic innervation in goats, Stroke, 19, 736-740

Tsubokawa, T. (1991) Calcium-dependent glutamate release concomitant with massive potassium flux during cerebral ischemia in vivo, Brain Research, 558, 136-140.

Tsuchida, E., Bullock, R. (1995) The effect of the glycine site-specific N-methyl-D-aspartate antagonist ACEA1021 on ischemic brain damage caused by acute subdural hematoma in the rat, Journal of Neurotrauma, 12, 279-288

Tsukahara, T., Usui, H., Taniguchi, T., Shimohama, S., Fujiwara, M., Handa, H. (1986) Characterisation of muscarinic cholinergic receptors in human and dog cerebral arteries, Stroke, 17, 300-305

Tuor, U., Kelly, P.A.T., Edvinsson, L., McCulloch, J. (1990) Neuropeptide Y and the cerebral circulation, Journal of Cerebral Blood Flow and Metabolism, 10, 591-601

Tuor, U., Kelly, P.A.T., Edvinsson, L., Tatemoto, K., McCulloch, J. (1985) Neuropeptide Y and the regulation of local cerebral blood flow, a quantitativeautoradiographic study, Journal of Cerebral Blood Flow and Metabolism, 5 (Suppl. 1), S513-S514

Turner, A.J. (1993) Endothelin-converting enzymes and other families of metalloendopeptidases, Biochemical Society Transactions, 21, 697-701

Tyson, G., Teasdale, G.M., Graham, D.I. , McCulloch, J. (1984) Focal cerebral ischemia in the rat, topography of hemodynamic and histopathological changes, Annals of Neurology, 15, 559-567.

Uddman, R., Edvinsson, L., Ekman, R., McCulloch, J., Kingman, T.A. (1985) Innervation of the feline cerebral vasculature by nerve fibres containing calcitonin gene-related peptide, trigeminal origin and co-existence with substance P, Neuroscience Letters, 62, 131-136

Uematsu, D., Greenberg, J.H., Hickey, W.F. , Reivich, M. (1989) Nimodipine attenuates both increase in cytosolic free calcium and histologic damage following focal cerebral ischemia and reperfusion in cats, Stroke, 20, 1531-1537.

Ulus, I.H., Arslan, B.Y., Savci, V., Kiran, B.K. (1995) Restoration of blood pressure by choline treatment in rats made hypotensive by haemorrhage, British Journal of Pharmacology, 116, 1911-1917

Umekawa, T., Matsumura, Y., Yoshimura, N., Murata, M., Takada, K., Tsukahara, Y., Takaoka, M., Morimoto, S. (1994) Platelets-induced stimulation of endothelin-1 production and inhibition by phosphoramidon, Journal of Pharmacology and Experimental Therapeutics, 269, 860-866

Umemura, K., Ishiye, M., Kosuge, K., Nakashima, M. (1995) Effect of combination of a tissue-type plasminogen activator and an endothelin receptor antagonist, FR139317, in the rat cerebral infarction model, European Journal of Pharmacology, 275, 17-21

Unterberg, A., Baethmann, A., Hack, U. (1983) Cerebral blood flow and metabolism during bradykinin exposure In, Cerebral Blood Flow, Metabolism and Epilepsy ed. Baldy-Moulinier M, Ingvar DH, Meldrum BD John Wiley, London pp 174-179

Unterberg, A., Wahl, M., Baethmann, A. (1984) Effects of bradykinin on permeability and diameter of pial vessels in vivo, Journal of Cerebral Blood Flow and Metabolism, 4, 574-585

Urade, Y., Fujitani, Y., Oda, K., Watakabe, T., Umemura, I., Takai, M., Okada, T., Sakata, K., Karaki, H. (1992) An endothelin B receptor selective antagonist, IRL1038 [Cys^{11} - Cys^{15}] endothelin-1 (11-21),

Velasco, C.E., Jackson, E.K., Morrow, J.A., Vitola, J.V., Inagami, T., Forman, M.B. (1993) Intravenous adnesine supresses cardiac release of endothelin after myocardial ischaemia and reperfusion, Cardiovascular Research, 27, 121-128

Vigne, P., Breittmayer, J.P., Marsault, R,. Frelin, C. (1991) Endothelins activate Na+/H+ exchange in brain capillary endothelial cells via a high affinity endothelin-3 receptor that is not coupled to phospholipase C, Journal of Biological Chemistry 266, 5925-5928

Vila, J.M., Martin de Aguilera, E., Martinez, M.C., Rodriquez, M.D., Iruazuni, A., Lluch, S. (1990) Endothelin action on goat cerebral arteries, Journal of Pharmacy and Pharmacology, 42, 370-372

Viossat, I., Duverger, D., Chapelat, M., Pirotsky, E., Chabrier, P.E., Braquet, P. (1993) Elevated tissue endothelin content during focal ischaemia in the rat, Journal of Cardiovascular Pharmacology, 22 (Suppl. 8), S306 - S309.

von Kummer, R., Herold, S. (1986) Hydrogen clearance method for determining local cerebral blood flow I. Spatial resolution, Journal of Cerebral Blood Flow and Metabolism, 6, 486-491

von Kummer, R., von Kries, F., Herold, S. (1986) Hydrogen clearance method determing local cerebral blood flow II. Effect of heterogeneity in cerebral blood flow, Journal of Cerebral Blood Flow and Metabolism, 6, 492-498

Wade, J.G., Amtrop, O., Sorensen, S.C. (1975) No-flow state following cerebral ischaemia. Role of increase in potassium concentration in brain interstitial fluid, Archives of Neurology, 32, 381 -384.

Wagner, O.F., Christ, G., Wojta, J. (1992) Polar secretion of endothelin-1 by cultured endothelial cells Journal of Biological Chemistry, 267, 16066-16068

Wahl M , Schilling L (1993) Mediators inducing stasis in cerebral microvessels during ischaemia in Microcirculatory Stasis in the Brain eds Tomita M et al pp 305-310

Wahl M, Unterberg A, Whalley ET, Baethmann A, Young AR, Edvinsson L, Wagner FFW (1986) Cerebrovascular effects of bradykinin In, Neural regulation of brain circulation ed. Owman C, Hardebo JE Elsevier, Amsterdam pp 415-430

Wahl, M., Deetjen, P., Thurau, K., Ingvar, D.H., Lasssen, N.A. (1970) Micropuncture evaluation of the importance of perivascular pH for the arteriolar diameter on the brain surface, Pflugers Archives, 316, 152-163

Wahl, M., Kuschinsky, W., Bosse, O., Olesen., J., Lassen, N.A., Ingvar, D.H., Michaelis, J., Thurau, K. (1972) Effect of L-norepinephrine on diameter of pial arterioles and arteries in the cat, Circulation Research, 31, 248-256

Wahl, M., Unterberg, A., Baethmann, A., Schilling, L. (1988) Mediators of blood-brain barrier dysfunction and formation of vasogenic brain edema, Journal of Cerebral Blood Flow and Metabolism, 8, 621-634

Wahl, M., Unterberg, A., Whalley, E.T. (1986) Cerebrovascular effects of bradykinin Fernstrom Foundation Series, 8, 419-430

Wahl, M., Young, A.R., Edvinsson, L., Wagner, F. (1983) Effects of bradykinin on pial arteries and arterioles in vitro and in situ, Journal of Cerebral Blood Flow and Metabolism, 3, 231-237

Wahlestedt, C., Edvinsson, L., Ekblad, E., Hakanson, R. (1985) Neuropeptide Y potentiates noradrenaline-evoked vasoconstrictions, mode of action, Journal of Pharmacology and Experimental Therapeutics, 234, 735-741

Wallace, J.L., Keenan, C.M., MacNaughton, W.K., McKnight, G.W. (1989) Comparison of the effects of endothelin-1 and endothelin-3 on the rat stomach, European Journal of Pharmacology, 167, 41-47

Walsh, T.F., Fitch, K.J., Toupence, R.B., Young, J.R., Williams, D.L., Nolan, N.A., O'Brein, J.A., Pettibone D.J., Kivlighn, S.D., Krause, S.M., Lis, E.V., Zingaro, G.J., Gabel, R.A., Hopkins, S., Clayton, F.C., Siegl, P.K.S., Greenlee, W.J. SAR and pharmacology of L751281, a member of a new series of non peptide ET antagonists (abstract no. C45) 4th International conference on Endothelin, (1995) 23-26 April; London U.K.

Wang, Q.D., Li, X.S., Lundberg, J.M., Pernow, J. (1995a) Protective effects of non-peptide endothelin receptor antagonist bosentan on myocardial ischaemic and reperfusion injury in the pig, Cardiovascular Research, 29, 805-812

Wang, Q.D., Uriuda, Y., Pernow, J., Hemsen, A., Sjoquist, P.O., Ryden, L. (1995b) Myocardial release of endothelin (ET) and enhanced ET(A) receptor-mediated

coronary vasoconstriction after coronary thrombosis and thrombolysis in pigs, Journal of Cardiovascular Pharmacology, 26, 770-776

Warner, T.D., Allcock, G.H., Corder, R., Vane, J.R. (1993) Use of the endothelin antagonists BQ-123 and PD-142893 to reveal 3 endothelin receptors mediating smooth-muscle contraction and the release of EDRF, British Journal of Pharmacology, 110,777-782.

Warner, T.D., Allcock, G.H., Mickley, E.J, Vane, J.R. (1993) Characterization of endothelin receptors mediating the effects of the endothelin/sarafotoxin peptides on autonomic neurotransmission in the rat vas deferens and guinea-pig ileum, British Journal of Pharmacology, 110, 783-789

Warner, T.D., Schmidt, H.H.H.W., Murad, F. (1992) Interactions of endothelins and EDRF in bovine native endothelial cells: selective effects of endothelin-3, American Journal of Physiology, H1600-H1605

Watanabe, T., Awane, Y., Ikeda, S., Fujiwara, S., Kubo, K., Kikuchi, T., Kusumoto, K., Wakimasu, M., Fujino, M. (1995) Pharmacology of a non selective ETA and ETB receptor antagonist TAK044 and the inhibition of myocardial infarct size in rats, British Journal of Pharmacology, 114, 949-954

Watanabe, T., Suzuki, N., Shimamoto, N., Fujino, M., Imada, A. (1990) Endothelin in myocardial infarction, Nature, 344,114.

Watanabe, T., Kusumoto, K, Kitayoshi, T., Shimamoto, N. (1989) Positive inotropic and vasoconstrictive effects of endothelin-1 in in vivo and in vitro experiments: characteristics and the role of L-type calcium channel, Journal of Cardiovascular Pharmacology, 13 (Suppl. 5), S108-S111

Watanabe, T., Suzuki, N., Shimamoto, N., Fujino, M., Imada, A. (1991) Contribution of endogenous endothelin to the extension of myocardial infarct size in rats, Circulation Research, 69, 370-377

Webb, M.L., Bird, J.E., Liu, E.C.K., Rose, P.M., Serafino, R., Stein, P., Moreland, S. (1995) BMS182874 is a selective non peptide endothelin ETA receptor antagonist, Journal of Pharmacology and Experimental Therapeutics, 272, 1124-1134

Wei, C.M., Lerman, A., Rodeheffer, R.J., McGregor, C.G.A., Brandt, R.R., Wright, S., Heublein, D.M., Kao, P.C., Edwards, W.D., Burnett, L.C. (1994) Endothelin in human congestive heart failure, Circulation, 89, 1580-1586

Wellings, R.P., Corder, R., Warner, T.D., Cristol, J.P., Thiemermann, C., Vane, J.R. (1994) Evidence from receptor antagonists of an important role for ET(B) receptor-mediated vasoconstrictor effects of endothelin-1in the rat kidney, British Journal of Pharmacology, 111, 515-520

Wendling, W.W., Harakal, C. (1991) Effects of prostaglandin F(2alpha) and thromboxane A2 analogue on bovine cerebral arterial tone and calcium fluxes, Stroke, 22, 66-72

Westfall T, Carpentier S, Chen X, Beinfeld MC, Naes L, Helddrum MJ (1987) Pre-junctional and post-junctional effects of neuropeptide Y at the noradrenergic neuroeffector junction of the perfused mesenteric arterial bed of the rat Journal Cardiovasc Res 10, 716-722

White, D.G., Cannon, T.R., Garratt, H. Mundin, J.W., Sumner, M.J., Watts, I.S. (1993) Endothelin ET(A) and ET(B) receptors mediate vascular smooth-muscle contraction, Journal of Cardiovascular Pharmacology, 22 [Suppl. 8], S144-S148

Willette, R., Sauermelch, C. (1990) Abluminal effects of endothelin in cerebral microvasculature assessed by laser doppler flowmetry, American Journal of Physiology, 259, H1688-H1693

Willette, R.N., Ohlstein, E.H., Pullen, M., Sauermelch, C.F., Cohen, A., Nambi, P. (1992) Transient forebrain ischaemia alters acutely endothelin receptor density and immunoreactivity in gerbil brain, Life Sciences, 52,35-40.

Willette, R.N., Zhang, H., Mitchell, M.P., Sauermelch, C.F., Ohlstein, E.H., Sulpizio, A.C. (1994) Non peptide enothelin antagonist, cerebrovascular characterisation and effects on delayed cerebral vasospasm, Stroke, 25, 2450-2456

Willette, R.N., Sauermelch, C., Ezekiel, M., Feuerstein, G., Ohlstein, E.H. (1990) Effect of endothelin on cortical microvascular perfusion in rats, Stroke, 21, 451-460

Williams, D.L., Nolan, N.A., O'Brein, J.A., Pettibone, D.J., Kivlighn, S.D., Krause, S.M., Lis, E.V., Zingaro, G.J., Gabel, R.A., Clayton, F.C., Siegl, P.K.S., Walsh, T.F., Fitch, K.J., Chakravarty, P.K., Greenlee, W.J. Pharmacology of L754142, a potent,

orally active nonpeptidyl endothelin antagonist (abstract no. C46) 4th International conference on Endothelin, (1995) 23-26 April; London U.K.

Winn, H.R,. Morii, S., Bern, R.M. (1985) The role of adenosine in autoregulation of cerebral blood flow, Annals of Biomedical Engineering, 13, 321-238

Winquist, R.J., Webb, R.C., Bohr, D.F. (1982) Relaxation to transmural nerve stimulation and exogenously added norepinephrine in porcine cerebral vessels, a study utilising cerebrovascular intrinsic tone, Circulation Research, 51, 769-773

Wolf, P.A., Abbott, R.D., Kannel, W.B. (1991) Atrial fibrillation as an independent risk factor for stroke, the Framingham study, Stroke, 22, 983-988

Wolf, P.A., Cobb, J.L., D'Agostino, R.B. (1992) Epidemiology of stroke In, Stroke, pathophysiology, diagnosis and management eds. Barnett HJM, Mohr JP, Stein BM, Yatsu FM Churchill Livingstone Inc., New York pp 3-27

Wu Wong, J.R., Chiou, W.J. Dixon, D.B., Opgenorth, T.J. (1995) Dissociation characteristics of endothelin ETA receptors agonists and antagonists, Journal of Cardiovascular Pharmacology, 26 (Suppl. 3), S380-S384

Wu Wong, J.R., Chiou, W.J. Magnuson, S.R., Opgenorth, T.J. (1994) Endothelin receptor agonists exhibit different dissociation characteristics, Biochemical and Biophysical Research Communications, 224, 288-294

Wyper, D.J., Sakas,D., Bullock, R., Patterson, J.L., Maxwell, W., Hadley, D., Teasdale, G.M. (1991) Traumatic cerebral contusions cause severely reduced peri-focal cerebral blood flow and ischaemic damage, Journal of Cerebral Blood Flow and Metabolism, 11 (Suppl. 1), S831

Xue, D., Huang, Z.-G., Barnes, K., Lesiuk, H.J., Smith, K.E. , Buchan, A.M. (1994) Delayed treatment with AMPA, but not NMDA, antagonists reduces neocortical infarction, Journal of Cerebral Blood Flow and Metabolism, 14, 251-261.

Yamasaki, H., Niwa, M., Yamashita, K., Kataoka, Y., Shigematsu, K., Hashiba, K., Ozaki, M. (1989) Specific 125I endothelin-1 binding sites in the atrioventricular node of the porcine heart, European Journal of Pharmacology, 168, 247-250

Yamashita, K., Kataoka, Y., Niwa, M., Shigematsu, K., Himeno, A., Koizumi, S., Taniyama, K. (1993) Increased production of endothelins in the hippocampus of

stroke prone spontaneously hypertensive rats following transient forebrain ischaemia, histochemical evidence, Cellular and Molecular Neurobiology, 13, 15-23.

Yamashita, K., Niwa, M., Kataoka, Y., Shigematsu, K., Himeno, A., Tsutsumi, K., Nakano-Nakashima, M., Sakurai-Yamashita, Y., Shibata, S., Taniyama, K. (1994) Microglia with an endothelin ETB receptor aggregate in rat hippocampus CA1 subfields following transient forebrain ischaemia., Journal of Neurochemistry 63, 1042-1051.

Yamashita, M., Kassell, N.F., Sasaki, T., Fujiwara, S., Zuccarello, M., Spallone, A. (1985) Topographical distribution of barrier function in cervico-cephalic arteries of dog, Stroke, 16, 875-879

Yamaura, I., Tani, E., Maeda, Y., Minami, N., Shindo, H. (1992) Endothelin-1 of canine basilar artery in vasospasm, Journal of Neurosurgery, 76, 99-105

Yamori, Y., Hori, R., Handa, H., Sato, M. , Fukase, M. (1976) Pathological similarity of strokes in stroke-prone spontaneously hypertensive rats and humans, Stroke, 7, 46-53.

Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K., Masaki, T. (1988) A novel potent vasoconstrictor peptide produced by vascular endothelial cells, Nature, 332,411-415.

Yang, Z.H., Richar, V., von Segesser, L., Bauer, E., Stulz, P., Turina, M., Luscher, T.F. (1990) Threshold concentrations of endothelin-1 potentiate contractions to norepinephrine and serotonin in human arteries: a new mechanism of vasospasm?, Circulation, 82, 188-195

Yip, P., He, Y., Hsu, C. (1991) Effect of plasma glucose on infarct size in focal cerebral ischemia-reperfusion., Neurology, 41,899-905.

Yoshimoto, S., Ishizaki, Y., Kurihara, M., Sasaki, T., Yoshizumi, M., Yanagisawa, M., Yazaki, Y., Masaki, T., Takahura, K., Murota, S. (1990) Cerebral microvessel endothelium is producing endothelin, Brain Research, 580, 283-285

Yoshinaga, M., Chijiiwa, Y., Misawa, T., Harada, N., Nawata, H. (1992) Endothelin B receptor on guinea pig small intestine smooth muscle cells, American Journal of Physiology, 262, G308-G311

Yoshizawa, T., Shinmi, O., Giaid, A., Yanagisawa, M., Gibson, S.T., Kimura, S., Uchiyama, T., Polak, J.M., Masaki, T., Kanazawa, I. (1990) Endothelin: a novel peptide in the posterior pituitary system, Science, 247, 462-464

Young, W. (1980) H_2 clearance measurement of blood flow, A review of technique and polargraphic principles, Stroke, 11, 552-564.

Zhang, R-L., Chopp, M., Chen, H., Garcia, J.H. , Zhang, Z.G. (1993) Postischemic (1 hour) hypothermia significantly reduces ischemic cell damage in rats subjected to 2 hours of middle cerebral artery occlusion, Stroke, 24, 1235-1240.

Zimmermann, R.S., Maymind, M., Barbee, R.W. (1994) Endothelin blockade lowers total peripheral resistance in haemorrhagic shock recovery, Hypertension, 23,205-210.

Zimmermann, M., Seifert, V., Stolke, D., Stenzel, W., Loffler, B-M. (1995) Ro47-0203 prevents cerebral vasospasm after experimental subarachnoid haemorrhage, Journal of Cerebral Blood Flow and Metabolism, 15[Suppl. 1], S703

Ziv, I., Heminger, G., Djaldetti, R., Achiron, A., Melamed, E., Sokolovsky, M. (1992) Increased plasma endothelin-1 in acute ischaemic stroke, Stroke, 23, 1014-1016

Zoja, C., Orisio, C., Perico, N., Benigni, A., Morigni, M., Benatti, L., Rambaldi, A., Remuzzi, G. (1991) Constitutive expression of endothelin gene in cultured human mesangial cells and its modulation by transforming growth factor-ß, thrombin and a thromboxane A2 analogue, Laboratory Investigation, 64, 16-20

Zuccarrello, M., Lewis, A.I., Rapoport, R.M. (1994) Endothelin ETA and ETB receptors in subarachnoid hemorrhage-induced cerebral vasospasm, European Journal of Pharmacology, 259, R1-R2

PUBLICATIONS

Peer Reviewed Journals:

Patel TR, McAuley MA, McCulloch J (1994) Effects on feline pial arterioles in situ of bosentan, a non-peptide, endothelin receptor antagonist *Eur J Pharmacol* 260: 65-71

Patel TR, Galbraith S, Graham DI, Hallak H, Doherty AM, McCulloch J (1996) Endothelin (ET_A) receptor antagonist increases cerebral perfusion and reduces ischaemic damage in feline focal cerebral ischaemia *J Cereb Blood Flow Metab* (in press)

Patel TR, Galbraith S, McAuley MA, McCulloch J (1996) Failure of endothelin receptor antagonist to alleviate post ischaemic hypoperfusion following transient global ischaemia in the rat *J Cereb Blood Flow Metab* (in press)

Patel TR, Galbraith S, McAuley MA, McCulloch J (1996) Increase in endothelin mediated vascular tone following feline focal cerebral ischaemia *J Cereb Blood Flow Metab* (in press)

Patel TR (1996) The therapeutic potential of endothelin receptor antagonists in cerebrovascular disease *CNS Drugs* (in press)

Patel TR, McAuley MA, McCulloch J Endothelin receptor mediated dilatation and constriction in feline cerebral resistance arterioles *in vivo* *Eur J Pharmacol* (in press)

Abstracts :

McAuley MA, Patel TR, Galbraith S, McCulloch J (1994) Endothelin and its pathophysiological role in the cerebral circulation in *Pharmacology of Cerebral Ischaemia* eds. Krieglstein, J. and Oberpichler- Schwenk, H. Wissenschaftliche Verlagsgesellschaft , Stuttgart *pp* 511-524

Patel TR, McAuley MA, McCulloch J (1994) The actions of bosentan , an endothelin receptor antagonist, on cerebral arterioles in situ *Br. J. Pharmacol.* 112: 486P

Patel TR, Galbraith S, McAuley MA, McCulloch J (1995) Cerebrovascular significance of endothelin receptor antagonism in focal ischaemia *J. Cereb. Blood Flow Metab. 15 [Suppl 1]* : S140

Patel TR, McCulloch J (1995) The role of endothelins in post ischaemic hypoperfusion following transient global ischaemia *J. Cereb. Blood Flow Metab. 15 [Suppl 1]* : S191

Patel TR, McAuley MA, Doherty AM, McCulloch J (1995) Pharmacological characterisation of peptide and non peptide endothelin receptor antagonists in cerebral arterioles in situ *J. Cereb. Blood Flow Metab. 15 [Suppl 1]* : S535

Patel TR, Smith CFC, Wilson C, McCulloch J (1995) Differences in the in vitro potencies of BQ-123 in antagonising contractile effects of endothelin-1 and endothelin-3 in the rabbit *J. Cereb. Blood Flow Metab. 15 [Suppl 1]* : S537

Patel TR, McAuley MA, McCulloch J (1995) Significance of endothelin ET_A and ET_B receptors in cerebral arterioles in situ *J. Cereb. Blood Flow Metab. 15 [Suppl 1]* : S542

Patel TR, McCulloch J (1995) Post-ischaemic hypoperfusion in transient global ischaemia - a role for endothelins? *J. Cardiovasc. Pharmacol.* 26 [Suppl. 3] : S425-S428

Patel TR, Galbraith SL, McAuley MA, Doherty AM, McCulloch J (1995) Therapeutic potential of endothelin receptor antagonists in experimental stroke *J. Cardiovasc. Pharmacol.* 26 [Suppl. 3] : S412-S415

GLASGOW
UNIVERSITY
LIBRARY