PEPTIDES AND THE CENTRAL REGULATION OF BLOOD PRESSURE

by

Moira A. McAuley

this being a thesis submitted for the degree of
Doctor of Philosophy
in the Faculty of Medicine
of the University of Glasgow

Department of Medicine and
Therapeutics

January 1990

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

TABLE OF CONTENTS

		Page no.
ACKNOWLEDGEMENTS		i
DECLARATION		iii
LIST OF TABLES		iv
LIST OF FIGURES		vi
SUMMARY		1
CHAPTER ONE - INTRODUCTION AND GENERAL BACKGROUND		6
1.1	**Brain Regions Involved in the Control of the Cardiovascular System - Overview**	8
1.2	**Neurotransmitters Associated with the Central Regulation of the Cardiovascular System - Overview of the Classical Transmitters and Neuropeptides**	12
1.2.1	Classical Transmitters	12
1.2.2	Neuropeptides	16
1.2.3	Cotransmission	20
1.3	**Importance of the Medulla Oblongata in the Control of the Cardiovascular System**	22
1.3.1	History	22
1.3.2	The Nucleus of the Solitary Tract	26
1.3.3	The Nucleus Ambiguus	30
1.3.4	The Rostral Ventrolateral Medulla	32
1.3.5	The Caudal Ventrolateral Medulla	37
1.4	**Second Messenger Systems Mediating Neurotransmitter or Hormonal Function**	41
1.4.1	Adenylate Cyclase System	42
1.4.2	Phosphoinositide Cycle	45
1.5	**Neuropeptide Y**	47

TABLE OF CONTENTS (Cont'd) Page no.

1.5.1	Isolation and Identification	47
1.5.2	Distribution in the Peripheral Nervous System	50
1.5.3	Distribution in the Central Nervous System	51
1.5.4	Effects in the Peripheral Nervous System	58
1.5.5	Effects in the Central Nervous System	62
1.5.6	Proposed Mechanisms of Action	65
1.6	Endothelin-1	66
1.6.1	Identification	66
1.6.2	Peripheral Distribution	68
1.6.3	Central Distribution	70
1.6.4	Effects in the Peripheral Nervous System	72
1.6.5	Effects in the Central Nervous System	75
1.6.6	Possible Mechanisms of Action	76
CHAPTER TWO - GENERAL METHODS AND MATERIALS		78
2.1	Introduction	79
2.2	Blood Pressure and Heart Rate Measurements in Chronically Anaesthetised Rats	79
2.3	Intraparenchymal Injections into the Medulla Oblongata of Anaesthetised Rats - Coordinates	80
2.4	Histology	81
2.5	Blood Pressure and Heart Rate Measurements in Conscious Rats	81
2.6	Intracisternal Cannulation	82
2.7	Determination of Plasma Catecholamine Levels	83
2.8	Measurement of cAMP Accumulation in Brain Slices	84

TABLE OF CONTENTS (Cont'd) Page no.

2.8.1	Preparation and Preincubation of Brain Slices	84
2.8.2	Drug Treatment of Slices	86
2.8.3	Measurement of cAMP Accumulation	86
2.8.4	cAMP Assay Protocol	87
2.8.5	cAMP Assay : Precision and Range	89
2.8.6	Preliminary Investigations	89
2.9	**Materials**	92
2.10	**Statistics**	93
CHAPTER THREE -	INDIVIDUAL ACTIONS OF NEUROPEPTIDE Y AND CLONIDINE AFTER MICROINJECTION INTO THE VENTROLATERAL MEDULLA	95
3.1	**Introduction**	96
3.1.1	Central Actions of Neuropeptide Y	96
3.1.2	Central Actions of Clonidine	99
3.2	**Methods**	101
3.2.1	Preparation of Animals for Intraparenchymal Microinjection Studies and Measurement of Arterial Pressure and Heart Rate	102
3.2.2	Statistical Analysis	102
3.3	**Results**	102
3.3.1	Control Responses in the Caudal and Rostral Ventrolateral Medulla	102
3.3.2	Effect of Neuropeptide Y Microinjected into the Caudal Ventrolateral Medulla	103
3.3.3	Effect of Neuropeptide Y Microinjected into the Rostral Ventrolateral Medulla	103
3.3.4	Effect of Clonidine Microinjected into the Caudal Ventrolateral Medulla	104

TABLE OF CONTENTS (Cont'd)

		Page no.
3.3.5	Effect of Clonidine Microinjected into the Rostral Ventrolateral Medulla	104
3.4	**Discussion**	105
3.4.1	Effects of Neuropeptide Y in the Ventrolateral Medulla	105
3.4.2	Effects of Clonidine in the Ventrolateral Medulla	110
CHAPTER FOUR - COADMINISTRATION OF NEUROPEPTIDE Y AND CLONIDINE IN THE VENTROLATERAL MEDULLA		116
4.1	**Introduction**	117
4.2	**Methods**	119
4.2.1	Coadministration of Neuropeptide Y and Clonidine in Rostral and Caudal Ventrolateral Medulla	119
4.2.2	Statistics	120
4.3	**Results**	120
4.3.1	Effect of Neuropeptide Y and Clonidine Coadministration in the Caudal Ventrolateral Medulla	120
4.3.2	Effect of Neuropeptide Y and Clonidine Coadministration in the Rostral Ventrolateral Medulla	121
4.4	**Discussion**	121
CHAPTER FIVE - EFFECT OF NEUROPEPTIDE Y ON cAMP LEVELS IN SLICES OF RAT MEDULLA OBLONGATA		125
5.1	**Introduction**	126
5.2	**Methods**	128
5.2.1	Preparation and Preincubation of Brain Slices	128
5.2.2	Drug Treatment of Slices	129
5.2.3	Pretreatment with Pertussis Toxin	129

TABLE OF CONTENTS (Cont'd) Page no.

5.2.4	Statistics	130
5.3	Results	130
5.3.1	Effect of Neuropeptide Y on Basal and Forskolin-stimulated cAMP Accumulation in the Medulla Oblongata	130
5.3.2	Effect of Neuropeptide Y on Basal and Forskolin-stimulated cAMP Accumulation in the Cortex	130
5.3.3	Effect of Pertussis Toxin Pretreatment on the Neuropeptide Y-mediated Inhibition of cAMP Accumulation in the Medulla Oblongata	131
5.4	Discussion	131
CHAPTER SIX - POSSIBLE INTERACTIONS BETWEEN NEUROPEPTIDE Y RECEPTORS AND ADRENOCEPTORS AT THE SECOND MESSENGER LEVEL		140
6.1	Introduction	141
6.2	Methods	143
6.2.1	Preparation and Preincubation of Brain Slices from the Medulla Oblongata	143
6.2.2	Drug Treatment of Slices - Alpha$_2$ Adrenergic Agonists	143
6.2.3	Drug Treatment of Slices - Beta-Adrenergic Agonists	143
6.2.4	Statistics	144
6.3	Results	144
6.3.1	Dose-response Relationship of Clonidine on Basal and Forskolin-stimulated cAMP Levels	144
6.3.2	Dose-response Relationship of Moxonidine on Basal and Forskolin-stimulated cAMP Levels	145
6.3.3	Possible Interaction Between the Effects of Moxonidine and Neuropeptide Y on Forskolin-stimulated cAMP Levels	145

TABLE OF CONTENTS (Cont'd) Page no.

6.3.4	Lack of Effect of Neuropeptide Y on Isoprenaline-stimulated cAMP Formation	145
6.4	Discussion	146
CHAPTER SEVEN - HAEMODYNAMIC EFFECTS OF ENDOTHELIN-1		154
7.1	The Haemodynamic Effects of Intravenous Administration of Endothelin-1 in the Anaesthetised and Conscious Rat	155
7.1.1	Introduction	155
7.1.2	Methods	156
7.1.2.1	Intravenous Administration in Anaesthetised Animals	156
7.1.2.2	Intravenous Administration in Conscious Animals	157
7.1.2.3	Measurement of Plasma Catecholamines	157
7.1.2.4	Statistical Analysis	157
7.1.3	Results	158
7.1.3.1	Effect of Intravenous Administration of Endothelin-1 on Blood Pressure and Heart Rate in the Anaesthetised Rat	158
7.1.3.2	Effects of Intravenous Administration of Endothelin-1 on Plasma Catecholamine Levels in the Anaesthetised Rat	158
7.1.3.3	Effect of Intravenous Administration of Endothelin-1 on Blood Pressure and Heart Rate in the Conscious Rat	159
7.1.3.4	Effect of Intravenous Administration of Endothelin-1 on Plasma Catecholamine in the Conscious Rat	161
7.1.4	Discussion	161
7.2	Haemodynamic Effects of Intracisternal Administration of Endothelin-1 in the Conscious Rat	166

TABLE OF CONTENTS (Cont'd) Page no.

7.2.1	Introduction	166
7.2.2	Methods	169
7.2.2.1	Preparation of Animals	169
7.2.2.2	Intracisternal Drug Administration	169
7.2.2.3	Plasma Catecholamine Determination	170
7.2.2.4	Statistical Analysis	170
7.2.3	Results	170
7.2.3.1	Effects of Intracisternal Administration of Endothelin-1 on Blood Pressure and Heart Rate	170
7.2.3.2	Effects of Intracisternal Administration of Porcins Endothelin-1 on Plasma Catecholamine Levels in the Conscious Rat	173
7.2.4	Discussion	173
7.3	Effect of Intracisternal Administration of Endothelin-1 on Cerebral Blood Flow in the Anaesthetised and Conscious Rat	178
7.3.1	Introduction	178
7.3.2	Measurement of Cerebral Blood Flow in Anaesthetised and Conscious Rats - Methodology	179
7.3.2.1	Preparation of Animals	179
7.3.2.2	Intracisternal Drug Administration	180
7.3.2.3	Cerebral Blood Flow Measurements with the [^{14}C]-Iodoantipyrine Autoradiographic Technique	181
7.3.2.4	Statistical Analysis	183
7.3.3	Results	183
7.3.3.1	Effect of Intracisternal Administration of ET-1 on Blood Pressure and Heart Rate in the Anaesthetised Rat	183

TABLE OF CONTENTS (Cont'd) Page no.

7.3.3.2	Effect of Intracisternal Administration of ET-1 on Cerebral Blood Flow in the Anaesthetised Rat	184
7.3.3.3	Effect of Intracisternal Administration of ET-1 on Blood Pressure and Heart Rate in the Conscious Rat	187
7.3.3.4	Effect of Intracisternal Administration of ET-1 on Cerebral Blood Flow in the Conscious Rat	188
7.3.4	Discussion	191
CHAPTER EIGHT - GENERAL DISCUSSION		197
PRESENTATIONS AND PUBLICATIONS CONTAINING THE WORK UNDERTAKEN FOR THIS THESIS		209
REFERENCES		210

ACKNOWLEDGEMENTS

I am indebted to Professor J.L. Reid for giving me the opportunity to study in his department and for the helpful discussions concerning my work.

To Dr. I.M. Macrae, many thanks for the sound advice, continual encouragement and for introducing me to Radio Scotland's Jimmy MacGregor!

I am also grateful to Professor J. McCulloch, Dr. M. Robinson and the technical staff of the Wellcome Surgical Institute for their collaboration in some of the studies contained within this thesis.

Kate Howie's expert statistical advice and the assistance of the Department of Medical Illustration, in particular Archie Shanks, in the production of various figures are also appreciated.

I am very grateful to Julie Nybo for the efficient typing of this thesis.

To Rosemary Farmer and Jim McCulloch, thank you for all the technical help etc. above and beyond the call of duty. I would like to thank my colleagues who are resident in, or have passed through (or passed out in!) the House on the Hill Office, whose banter made those long days shorter.

I would also like to thank my friends who have been a source of a variety of things including biscuits and many an ear during my 'writing period'.

Finally, a special acknowledgement and thanks must go to my family, especially to my Mum and Dad for their constant support, encouragement and funding throughout my undergraduate and postgraduate years.

DECLARATION

I declare that this thesis has been composed by myself and is a record of work performed by myself. It has not been submitted previously for a higher degree.

This research was carried out in the Department of Medicine and Therapeutics, University of Glasgow, under the supervision of Dr. I.M. Macrae and Professor J.L. Reid.

January, 1990 M.A. McAuley

LIST OF TABLES

		Page no.
Table 1.1	A list of peptides with established or putative influences on central cardiovascular function-selected examples	18
Table 1.2	Coexistence of classical transmitters and peptides in the central nervous system - selected examples	23
Table 1.3	Amino acid sequences of neuropeptide Y and three cognate peptides	49
Table 1.4	Distribution of neuropeptide Y-like immunoreactivity within regions of the rat central nervous system	53
Table 1.5	Summary of differential coexistence patterns of neuropeptide Y-like immunoreactivity within regions of the rat central nervous system	56
Table 1.6	Reported peripheral effects of neuropeptide Y	59
Table 1.7	Reported central effects of neuropeptide Y	64
Table 1.8	Autoradiographic distribution of (^{125}I)-ET-1 binding sites in selected regions of the rat and human brain	71
Table 2.1	Composition of buffer used in the incubation of brain slices	85
Table 2.2	cAMP Assay Protocol	88
Table 2.3	Typical Assay Data	90
Table 3.1	Proposed sites of the antihypertensive action of clonidine in the central nervous system	100
Table 7.1	Lack of effect of intravenous endothelin-1 on plasma catecholamine levels in the anaesthetised rat	160

LIST OF TABLES (Cont'd) Page no.

Table 7.2	Lack of effect of intravenous administration of endothelin on plasma catecholamine levels in the conscious rat	162
Table 7.3	Individual blood pressure responses and corresponding heart rate values before and at the maximum response following intracisternal endothelin-1 in the conscious rat	173
Table 7.4	The effect of intracisternal endothelin-1 on blood pressure and heart rate in the anaesthetised rat	185
Table 7.5	The effect of intracisternal endothelin-1 on cerebral blood flow in the anaesthetised rat	186
Table 7.6	The effect of intracisternal endothelin-1 on mean arterial pressure and heart rate in the conscious rat	189
Table 7.7	The effect of intracisternal endothelin-1 on cerebral blood flow in the conscious rat	190

LIST OF FIGURES

Figure 1.1 Sagittal cross-section of the rat brain showing important brain regions known to be involved in cardiovascular control

Figure 1.2 Schematic cross-section of the rat brain showing the location of noradrenaline, adrenaline and dopamine cell groups

Figure 1.3 Schematic diagram showing the location of catecholamine cell groups in the medulla involved in the control of blood pressure and heart rate

Figure 1.4 Dual regulation of adenylate cyclase

Figure 1.5 Receptor-mediated phosphionositide turnover

Figure 1.6 Amino acid sequences of peptides from the endothelin family

CHAPTER TWO

Figure 2.1 Diagram illustrating the site of microinjection in the caudal ventrolateral medulla of the rat

Figure 2.2 Diagram illustrating the site of microinjection in the rostral ventrolateral medulla of the rat

Figure 2.3 Diagram showing the dimensions of the intracisternal cannula and stylette

Figure 2.4 General protocol for the incubation of rat brain slices

Figure 2.5 A typical cAMP standard curve

Figure 2.6 Dose-response graph of forskolin (10^{-8} -10^{-5}M) in slices of the rat medulla oblongata

Figure 2.7 Time-response graph of forskolin (10^{-5}M) in slices of the rat medulla oblongata

CHAPTER THREE

Figure 3.1 Haemodynamic effects of neuropeptide Y microinjections into the caudal ventrolateral medulla

LIST OF FIGURES (Cont'd)

Figure 3.2 Haemodynamic effects of neuropeptide Y microinjections into the rostral ventrolateral medulla

Figure 3.3 Haemodynamic effects of clonidine microinjections into the caudal ventrolateral medulla

Figure 3.4 Haemodynamic effects of clonidine microinjections into the rostral ventrolateral medulla

CHAPTER FOUR

Figure 4.1 Haemodynamic effects of coadministration of neuropeptide Y (25 pmol) together with clonidine (10 nmol) into the caudal ventrolateral medulla

Figure 4.2 Haemodynamic effects of coadministration of neuropeptide Y (50 pmol) together with clonidine (10 nmol) into the caudal ventrolateral medulla

Figure 4.3 Haemodynamic effects of coadministration of neuropeptide Y (25 pmol) together with clonidine (10 nmol) into the rostral ventrolateral medulla

CHAPTER FIVE

Figure 5.1 Structure of the diterpene forskolin

Figure 5.2 Protocol for the investigation of the effect of neuropeptide Y on basal and forskolin-stimulated cAMP levels in the rat medulla oblongata

Figure 5.3 Effect of neuropeptide Y on basal and forskolin-stimulated cAMP levels in the rat medulla oblongata

Figure 5.4 Effect of neuropeptide Y on basal and forskolin-stimulated cAMP levels in the rat cortex

Figure 5.5 Effect of pertussis toxin pretreatment on the neuropeptide Y-mediated inhibition of cAMP accumulation in the medulla oblongata

CHAPTER SIX

Figure 6.1 Protocol for the investigation of the effect of clonidine on basal or forskolin-stimulated cAMP levels in the medulla oblongata

LIST OF FIGURES (Cont'd)

Figure 6.2 Protocol for the investigation of the effect of neuropeptide Y or propranol on basal or isoprenaline-stimulated cAMP levels in the medulla oblongata

Figure 6.3 Lack of effect clonidine on basal cAMP levels in the rat medulla oblongata

Figure 6.4 Lack of effect of clonidine on basal and forskolin-stimulated cAMP levels in the medulla oblongata

Figure 6.5 Lack of effect of moxonidine on basal cAMP levels in the rat medulla oblongata

Figure 6.6 Effect of moxonidine on basal and forskolin-stimulated cAMP levels in the rat medulla oblongata

Figure 6.7 Effect of moxonidine together with neuropeptide Y on basal and forskolin-stimulated cAMP levels in the medulla oblongata

Figure 6.8 Effect of isoprenaline in the absence and presence of propranolol on cAMP levels in the medulla oblongata

Figure 6.9 Lack of effect of NPY on basal and isoprenaline-stimulated cAMP levels in the medulla oblongata

Figure 6.10 Structure of the $alpha_2$-adrenoceptor agonist clonidine

Figure 6.11 Structure of the $alpha_2$-adrenoceptor agonist moxonidine

CHAPTER SEVEN

Figure 7.1 The effects of intravenous endothelin-1 on the blood pressure and heart rate of the anaesthetised rat

Figure 7.2 Representative trace of the arterial blood pressure and heart rate following intravenous endothelin-1 in the anaesthetised rat

LIST OF FIGURES (Cont'd)

Figure 7.3 The effect of intravenous endothelin-1 on blood pressure and heart rate in the conscious rat

Figure 7.4 Representative trace showing arterial blood pressure and heart rate following intravenous endothelin-1 in the conscious rat

Figure 7.5 The effect of intracisternal endothelin-1 on blood pressure and heart rate in the conscious rat

Figure 7.6 Representative trace of the arterial blood pressure and heart rate following intracisternal endothelin-1 in the conscious rat

Figure 7.7 The effect of intracisternal endothelin-1 on plasma catecholamine levels in the conscious rat

Figure 7.8 Operational equation to determine cerebral blood flow using the [^{14}C]-Iodoantipyrine autoradiographic technique

Figure 7.9 Individual blood pressure responses and corresponding heart rate values before and at the maximum response to intracisternal endothelin-1 or saline in the anaesthetised rat

Figure 7.10 Individual blood pressure responses and corresponding heart rate values before and at the maximum response following intracisternal injection of ET-1 or saline in the conscious rat

Figure 7.11 Representative autoradiographs of cerebral blood flow in saline and endothelin-1 treated animals

SUMMARY

It is now recognised that a vast number of peptides are present in the central nervous system and that like classical transmitters some of these peptides may participate in the central regulation of the cardiovascular system. These neuropeptides may be colocalised in neurones with other transmitters, such as catecholamines, in brain areas which include the important cardiovascular regulatory region, the medulla oblongata. The peptides reputedly act either independently or they undertake a neuromodulatory role where they may interact with the function of the costored transmitters. Delineation of the cardiovascular actions and possible interactions of peptides found in regions of cardiovascular significance may assist in the understanding of the labyrinthe mechanisms involved in centrally-mediated haemodynamics.

In this respect the first series of investigations (Chapter Three) examined the potential cardiovascular role of neuropeptide Y (NPY). Previous immunohistochemical studies have demonstrated the presence of this peptide in many brain regions. Of particular interest was the existence of NPY-immunoreactive cell bodies and terminals in two regions of the medulla oblongata, namely, the A_1 noradrenergic cell group in the caudal ventrolateral medulla and the C_1 adrenergic cell group of the rostral ventrolateral medulla. These regions are of considerable haemodynamic importance since they participate in the tonic and reflex regulation of blood pressure. Discrete microinjection of NPY (25 and 50 pmol) in the caudal region of the anaesthetised

rat produced a dose-related decrease in blood pressure. It is proposed that in the caudal region NPY may be involved in the mechanisms controlling blood pressure and that this peptide stimulates the A_1 (vasodepressor) cell group to elicit the observed haemodynamic response. A fall in heart rate also proceeded the injection of NPY into this area. This response was not dose-related, and may indicate that a maximum response had already been reached at the lower dose. If this is the case, it would suggest that the mechanisms involved in the regulation of heart rate in this region are more sensitive to NPY than those of blood pressure, resulting in a similar magnitude of bradycardia at both doses of the peptide. Injection of NPY (25 and 50 pmol) into the more rostral (C_1) area did not evoke any significant haemodynamic response.

The functional implications of the reported coexistence of NPY with catecholamines in the A_1 and C_1 cell groups were investigated in Chapter Four. It has been purported that the endogenous catecholamines of these regions (noradrenaline in the A_1 region and adrenaline in the C_1 region) alter cardiovascular function principally through α_2-adrenoceptors therefore the possible interaction between NPY receptors and α_2-adrenoceptors was investigated by simultaneous injection of NPY and the α_2-adrenoceptor agonist clonidine into these discrete regions. Clonidine (10 and 20 nmol) alone evoked a dose-related hypotension and bradycardia in both the caudal and rostral ventrolateral medulla (Chapter Three). Coadministration of NPY

(25 or 50 pmol) together with a submaximal dose of clonidine (10 nmol) in these regions however, produced a hypotensive response which was comparable to the sum of their individual responses. These results indicate that although NPY and catecholamines have been shown to be colocalised in the caudal and rostral ventrolateral medulla a functional interaction pertaining to blood pressure regulation was not evident. The significance of their costorage with regard to this haemodynamic parameter may therefore reflect a provision for separate functions which are required or evoked under different physiological conditions. It is interesting that in the caudal but not the rostral area, the coadministration of NPY (25 pmol) and clonidine (10 nmol) elicited a fall in heart rate which was significantly less than the sum of the individual heart rate responses.

Chapters Five and Six deal with the molecular mechanisms involved in the central effects of NPY. In particular, the effect of NPY on cAMP levels in slices of the rat medulla oblongata was examined, since studies in other tissues have indicated that the functioning of this second messenger system was altered by NPY. In agreement with such studies, NPY was shown to reduce the forskolin-stimulated cAMP levels in slices of the medulla oblongata and cortex. Moreover the ability of NPY to inhibit forskolin-stimulated cAMP levels in medullary slices was abolished subsequent to intracisternal pretreatment with pertussis toxin. These results support the involvement of the cAMP system in the central actions of NPY, since this toxin is

known to inactivate a G-protein necessary for receptor-mediated reduction in adenylate cyclase activity and consequently cAMP levels.

Although interactions between the haemodynamic responses to NPY and the α_2-adrenoceptor agonist clonidine were generally absent in the ventrolateral medulla, one study has observed a functional interaction between NPY receptors and α_2-adrenoceptors in another important region of the medulla, namely, the nucleus of the solitary tract. The next series of experiments therefore examined the possibility of an interaction being evident in the medulla oblongata between NPY and α_2-adrenoceptors at the level of the cAMP system. The effects of α_2-adrenoceptor agonists alone were initially investigated. Clonidine did not significantly alter the levels of forskolin-stimulated cAMP in slices of the medulla whereas another antihypertensive agent moxonidine, with more selective α_2-adrenoceptor properties, produced a significant reduction in this parameter. Further studies also revealed that the inhibitory action of NPY on forskolin-stimulated cAMP levels was attenuated in the presence of moxonidine suggesting the existence of an antagonistic interaction between the respective receptors. An interaction between NPY and β-adrenoceptors at the level of the cAMP system was not apparent in the present study.

In the final chapter, the central haemodynamic actions of the recently discovered peptide endothelin-1 (ET-1) were investigated. Intracisternal administration of ET-1 (0.01 -

0.03 nmol) in the conscious rat produced a marked haemodynamic response at the highest doses of the peptide studied. A pronounced increase in blood pressure was observed accompanied in some cases by intense motor activity, but no consistant change in heart rate. The cardiovascular profile following this route of administration differed qualitatively from the response obtained following intravenous administration of the peptide. Furthermore, a haemodynamic response was elicited at lower doses of ET-1 subsequent to intracisternal administration of ET-1 than those necessary to produce a response following intravenous administration. These data would suggest that the haemodynamic response following was centrally-mediated and not due to leakage of the peptide into the peripheral nervous system. The hypothesis that the hypertensive event observed after intracisternal ET-1 administration was due to an action on cerebral blood vessels rather than an action on brain parenchyma was also investigated in this final chapter. Regional cerebral blood flow was measured using the [^{14}C]-Iodoantipyrene autoradiographic technique and the data obtained clearly demonstrated that the dramatic increase in blood pressure after intracisternal ET-1 (0.03 nmol) administration in the conscious rat was associated with a widespread and profound reduction in cerebral blood flow throughout the medulla. Although an action of ET-1 on central neural mechanisms regulating vasomotor outflow cannot be excluded, it is propounded that the first line of action of this peptide following intracisternal administration occurs at the level of the cerebral vasculature.

CHAPTER ONE

INTRODUCTION AND GENERAL BACKGROUND

Chapter One

Introduction and General Background

In the attempts to understand the mechanisms underlying the central control of blood pressure much attention has been focused on the role of the medulla oblongata. This extensive field of research has included identifying the neurotransmitters present within this brain region. Indeed the recognition that catecholamine neurone groups exist in the medulla oblongata and are associated with blood pressure regulation, has formed a basis for efforts to examine the function of other putative transmitters in this region. The recent discovery of a pervasive distribution of peptides in the mammalian nervous system has raised the question of their functional significance. Encouraged in some cases by haemodynamic studies in the peripheral nervous system, evidence has now accumulated that several peptides may participate in central cardiovascular regulatory mechanisms. In this respect two newly identified peptides, neuropeptide Y and endothelin-1, both with documented evidence of haemodynamic actions in the peripheral nervous system will be considered, with the aim of examining their possible centrally-mediated cardiovascular activities. In particular, attention will be centred on the effects and mode of action of neuropeptide Y in the medulla oblongata. In addition the central haemodynamic actions of endothelin-1 and its possible role in cerebrovascular function will also be examined. As a milieu to this work the organisation of the central nervous system with regard to the

regulation of blood pressure will be briefly discussed in this first chapter, followed by an overview of the central transmitters and peptides considered important in the central neural control of the cardiovascular system. The role of the medulla oblongata will then be examined in more detail concentrating on the neurocircuitry and transmitters associated with the various nuclei of this brain region. Next the second messengers which may serve as the final biochemical end-point for the action of these and other putative neurotransmitters will be reviewed. Finally the short history of the two peptides neuropeptide Y and endothelin will be discussed with reference to their peripheral as well as central distribution and activities.

1.1 Brain Regions Involved in the Control of the Cardiovascular System-overview

It is now well appreciated that neural and hormonal systems at all levels of the central nervous system participate in the maintenance of blood pressure homeostasis. Thus, various portions of the central neuroaxis are recognised as able to regulate the function of the heart (cardiac output), blood vessels (peripheral resistance) and consequently blood pressure, by the release of key humoral factors in concert with altering the level of activity conveyed over the parasympathetic and sympathetic branches of the autonomic nervous system.

The location of the general regions associated with cardiovascular control are shown in Figure 1.1 and will be discussed briefly.

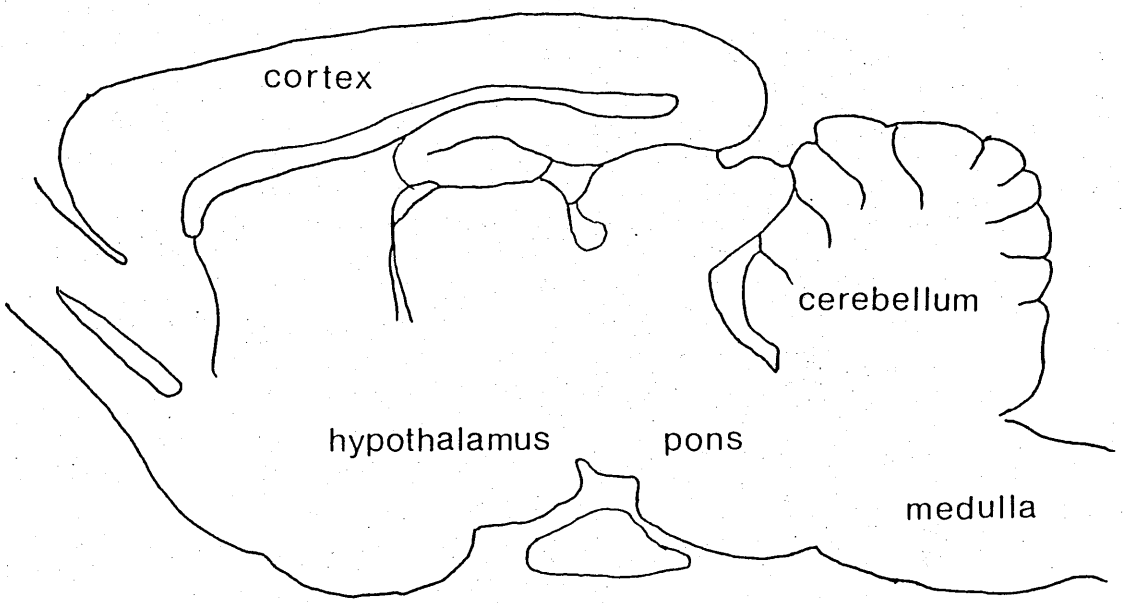


Figure 1.1

Sagittal cross-section of the rat brain showing important brain areas known to be involved in cardio-vascular control

The brainstem, which comprises the medulla oblongata and pons, can be regarded as the most caudal link in the series of longitudinal systems extending from the cerebral cortex which are involved in this central control of the cardiovascular system. The medullary portion of the brainstem contains the nucleus of the solitary tract which serves as the major terminus for afferent input, from systemic baroreceptors and chemoreceptors, concerning the state of the cardiovascular system. This nucleus integrates and then relays this information to a wide variety of regions including the ventrolateral aspects of the medulla (Howe, 1985). Axons from neurones in the latter area descend in the intermediolateral cell columns of the spinal cord to innervate the sympathetic preganglionic neurones situated in the spinal cord (Ross et al, 1981a). The balance of stimulation and inhibition provides the control of vasomotor tone by modulating the activity of the preganglionic neurones. These neurones are known to project to the paravertebral ganglia where they regulate the activity of the sympathetic postganglionic neurones which innervate the heart and blood vessels. Stimulation of these nerves causes vasoconstriction of resistance vessels and veins, vasodilation largely being achieved by fewer vasoconstrictor impulses. Most of the parasympathetic (vagal) fibres which influence heart rate and balance sympathetic tone to the heart originate in a region of the medulla termed the nucleus ambiguus. This nucleus receives an input from and is influenced by the nucleus of the solitary tract (Thomas and Calaresu, 1974). The paramedian reticular nucleus of the medulla as well as the

fastigial nucleus of the cerebellum receive communication from the vestibular apparatus and make up the network responsible for changes in sympathetic tone during changes in posture (Miura and Reis, 1971). The area postrema, a circumventricular organ located in the caudal medulla, also reputedly influences the central neural output to cardiovascular effectors. This important area receives afferent input from cardiovascular receptors and projects to brain regions associated with cardiovascular control including the nucleus of the solitary tract and the parabrachial nucleus (Shapiro and Miselis, 1985). It is devoid of a blood brain barrier and this allows neural elements within and perhaps subjacent to this region to be directly exposed to circulating hormones such as vasopressin and angiotensin. The more rostrally located parabrachial nucleus of the pons also plays a significant role in the neural and humoral modulation of the cardiovascular system. This nucleus has widespread connections with other cardiovascular centres, in particular the nucleus of the solitary tract and the hypothalamus and when stimulated evokes an increase in arterial pressure and heart rate (Mraovitch et al, 1982).

The hypothalamus, and related structures which lie along the lamina terminalis, have been implicated as sites of higher cardiovascular control for a number of years. These areas provide for the integration of somatic, endocrine and autonomic functions and can modulate these functions according to emotional situations. Activation of the various hypothalamic centres

results in a myraid of vasopressor and depressor states, some of which are closely involved with the activity of the important cardiovascular nuclei in the medulla oblongata. These pathways therefore provide a means for higher centres to conduct cardiovascular regulation. Electrical stimulation of the paraventricular nucleus, anterior (AV3V), or posterior hypothalamic regions evoke an increase in blood pressure. In the latter region an inhibitory action on baroreceptor reflexes was also observed and these effects have been associated with the preparation for the 'flight or fight' reaction (Cirello and Calaresu, 1980a; Brody et al, 1980). In contrast, stimulation of the dorsal anterior hypothalamic region appears to elicit a vasodepressor response (Gauther et al, 1981).

Higher centres, such as the cerebral cortex have been shown to be involved in conditioned and learned cardiovascular responses. It is possible that cortical perceptions such as stress can be transmitted to centres responsible for cardiovascular regulation and may chronically influence blood pressure (Ganten and Pfaff, 1983). A close anatomical and functional integration between the hypothalamic and cortical regions is provided by the limbic system. This area is particularly associated with cardiovascular adaptation in response to fear and rage.

1.2 Neurotransmitter Associated with the Central Regulation of the Cardiovascular System - Overview of the Classical Transmitters and Neuropeptides

1.2.1 Classical Transmitters

Although the participation of humoral mechanisms in the central control of blood pressure has been recognised since the beginning of the century, the knowledge that processing in the brain involves communication among neurones - through the release of chemical messengers at synapses - was only established with certainty much later (Eccles, 1954). Acetylcholine was the only generally accepted transmitter in the central nervous system for some time, however, with the development of histochemical and neuroanatomical techniques, a fairly rapid increase in the number of candidates ensued. The histochemical fluorescence studies of Dahlstom and Fuxe (1964; 1965) demonstrated the presence of discrete collections of neurones containing catecholamines. This supported earlier biochemical studies which suggested such a role for noradrenaline and dopamine in central neurotransmission (Vogt, 1954; Carlsson et al, 1958).

Histochemical analysis further revealed that noradrenergic cell groups were located in the brainstem and these were subsequently labelled A_1 - A_7 (Figure 1.2). It was considered at that time that the most caudally situated noradrenergic cell groups in the ventrolateral and dorsomedial medulla (A_1/A_2) provided the descending projections to the spinal cord whereas

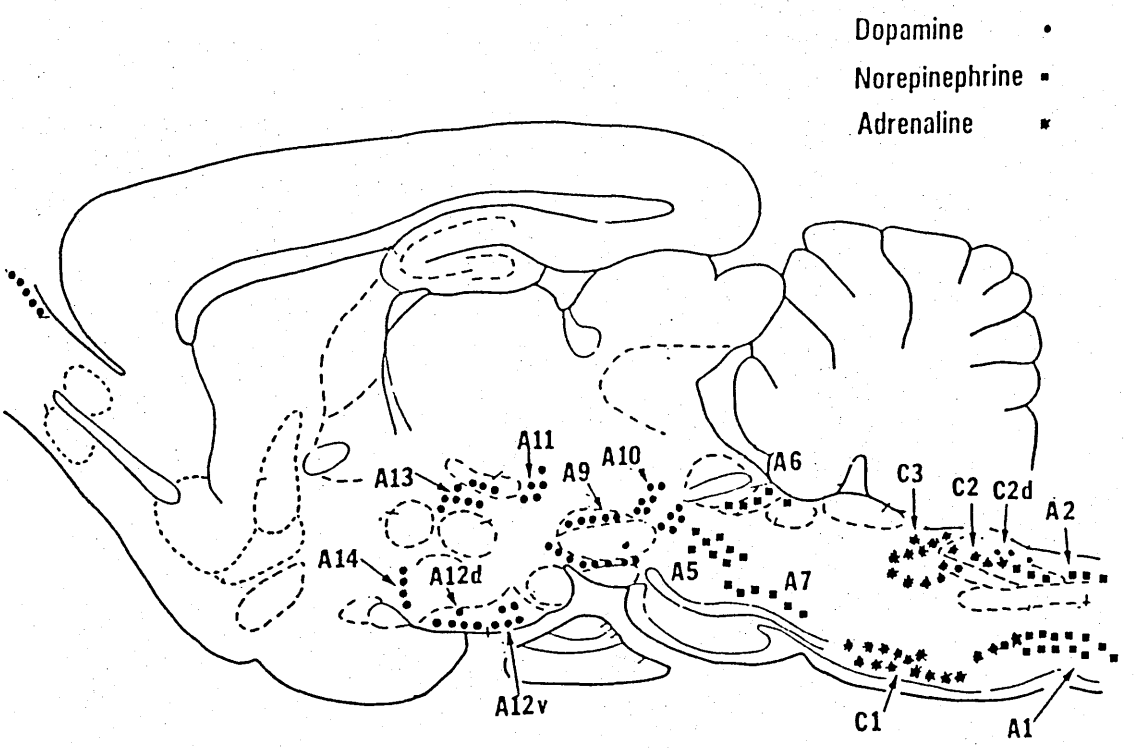


Figure 1.2

Schematic drawing of a sagittal section of rat brain showing the location of noradrenaline, adrenaline and dopamine cell groups.

The nomenclature of the different cell groups according to Dahlstrom et al (1964; 1965); Hokfelt et al (1974).

the other rostral groups, for instance the A6 group in the pontine locus coeruleus, ascended to more rostral areas to terminate in the cerebral hemispheres, the cerebellum and the hypothalamus. Dopamine (A_8 - A_{16}) cells were found mainly in mid and rostral parts of the brain in areas such as the mesencephalon, the hypothalamus, and the olfactory bulb, with scattered cells in other brain regions (Figure 1.2). Serotonin neurones were also described by these authors with descending spinal neurones arising from the B_1 - B_3 groups in the medulla and the more rostrally located cell groups (B_4 - B_9) providing ascending projections. The later discovery of adrenaline in central neurones led to the modification of the original descriptions by Dahlstrom and colleagues. Hokfelt et al (1974), using immunofluorescence to demonstrate the presence of the enzyme phenylethanolamine-N-methyltransferase (PNMT) which catalyses the conversion of noradrenaline to adrenaline established the presence of 2 medullary groups of adrenaline cells. These neurones designated C_1 and C_2 were situated in the ventrolateral and dorsomedial medulla within the rostral portions of the noradrenergic A_1 and A_2 cell groups, respectively (Figure 1.2).

Thus it was postulated that central neurones could utilise adrenaline as well as noradrenaline and dopamine as neurotransmitters. Although a third group of adrenaline-containing neurones and a dopamine cell group were described recently in the medulla by Howe et al (1980) and Armstrong et al (1982) respectively, a more significant modification to the

orginal work of Dahlstrom and Fuxe stemmed from the development of neuroanatomical techniques such as the retrograde transport of horseradish peroxidase to map central pathways. It was realised that projections from the catecholamine cell groups were more complicated than originally envisaged with some of the pathways long and often diffuse. Ascending pathways were shown from the most caudal noradrenergic groups (A_1/A_2) as well as descending projections from the more rostral nuclei. Moreover some investigators now believed that the descending catecholamine projections did not arise exclusively (if at all) from the noradrenergic A_1/A_2 located in the medulla but that more rostrally located medullary neurones including the adrenergic C_1 cell group and the serotonergic B_1, B_2 and B_3 provided that innervation (Ross et al, 1981a; Dahlstrom and Fuxe, 1964; 1965). At the level of the medulla oblongata, the main adrenergic projection group is the C_1 group which was shown to give rise to both ascending and descending pathways. The ascending adrenergic pathways may run in the medullary catecholamine bundle or as recent studies suggest more ventrally along the ventromedial edge of the brainstem (Palkovits and Brownstein, 1989). The descending spinal adrenergic projections run in the dorsal part of the lateral funiculus and innervate almost exclusively the lateral sympathetic column.

Axons of the medullary noradrenergic neurones run in the medullary catecholamine bundle (also known as the tegmental tract) which carries both ascending axons from the A_1 and A_2

groups and descending axons from the A_6 and A_5 cell groups (Palkovits and Brownstein, 1989; Hokfelt et al, 1974).

It is of considerable interest that the distribution of these central catecholamine neurones, in particular those located in the brainstem, are similar to the location of the central regions and pathways involved in cardiovascular regulation (Figure 1.1, Chalmers, 1975) - a concept that will be discussed in more detail in Section 1.3. Moreover it has been demonstrated that chemical inhibition or destruction of central catecholaminergic or serotonergic nerves has marked effects on blood pressure (Haeusler et al, 1972; Saavedra et al, 1976; Wing and Chalmers, 1974). While these central monaminergic systems apparently participate in the regulation of blood pressure under normal conditions, it has also been shown that their function may be altered under conditions of high blood pressure. Thus, changes in the metabolism and activity of these monoamine neurones have been reported in a number of models of experimental hypertension suggesting that these transmitters are involved in some way either in the generation of, or response to hypertensive states (Nakamura et al, 1971; Wing and Chalmers, 1974; Reid and Rubin, 1989).

Amino acids came into focus in the 1970s as potential inhibitory and excitatory transmitters in the central nervous system. As reviewed by Hokfelt et al (1984b) autoradiographical studies describe the presence of the amino acids gamma-aminobutyric acid (GABA), glycine and glutamate within neuronal systems. Quantitative and immunohistochemical studies have

further confirmed that extensive neuronal systems containing the inhibitory amino acid GABA exist in the central nervous system. While some of these GABA neurones have long projections, for instance from the posterior hypothalamus to cortical or subcortical areas, a large majority of these systems represent local neuronal networks (Jessell et al, 1978). These interneurones have been observed in the ventrolateral medulla where pharmacological evidence suggests that they play a tonic inhibitory role in the regulation of blood pressure (Ruggiero et al, 1985a; Reis, 1987). A pressor action of glutamate has been observed in the medulla. Indeed this putative transmitter has been proposed as a candidate for excitatory input to cardiovascular regulation at various levels of the medulla including the NTS and the C_1 regions (Sun et al, 1986).

1.2.2 Neuropeptides

In addition to the 'classical transmitters' described above an explosion in the number of possible chemical messengers resulted from the appreciation that peptides were present in central neurones and may also serve a neurotransmitter or neuromodulator function. Attempts to identify the neuropeptides responsible for centrally-mediated cardiovascular functions are impeded by their sheer number and pervasive distribution. Moreover complications arise from the finding that a peptide may have varied effects on a particular function at different levels within the central nervous system. Indeed elucidation of their respective roles has often followed their neuroanatomical

localisation. While many of these peptides fulfil some of the essential criteria suggested by Burnstock et al (1979) for establishing chemical messengers as neurotransmitters, most peptides have not yet been tested for all of these criteria. However, a number of peptides with established or putative central cardiovascular regulatory functions have been described in the last few years following biochemical, immunohistochemical and physiological investigations and have been reviewed very recently by Reid and Rubin (1987) and Gardiner and Bennett (1989). Some of these putative neuropeptides are shown in Table 1.1. While it is outside the scope of this discourse to consider all of these peptides, the central cardiovascular significance of a selected few will be discussed.

The opioid peptides methionine-enkephalin and leucine-enkephalin have attracted considerable attention not least because of their wide central distribution. Another opioid peptide β-endorphin is also extensively located but is distinct from the enkephalin peptides (Lang et al, 1984). The hypothalamus and brainstem, cardiovascular regulatory regions, contain substantial amounts of opioid peptides. However, central administration of these peptides has revealed contradictory results with pressor and depressor responses observed depending on the area, species and specific peptide investigated. In general, leucine and methionine-enkephalin appear to evoke a central pressor response in conscious animals although depressor effects have been described in anaesthetised animals (Schaz

Table 1.1

A List of Peptides with Established or Putative Influences on Central Cardiovascular Control - Selected Examples

β-endorphin

Leucine-enkephalin

Methionine-enkephalin

Angiotensin II

Vasopressin

Oxytocin

Corticotrophin-releasing hormone

Thyrotrophin-releasing hormone

Somatostatin

Substance P

Bradykinin

Neurotensin

Galanin

Neuropeptide Y

Atrial natriuretic factor

Vasoactive intestinal polypeptide

Calcitonin-gene related peptide

Bombesin

Endothelin (?)

Data taken from Gardiner and Bennett (1989); Reid and Rubin (1987); Yanagisawa and Masaki (1989a).

et al, 1980; Yakimura et al, 1981). β-endorphin elicits primarily a hypotensive effect when administered centrally. In particular this peptide has a depressor effect in the medulla and within this area may participate in the reflex regulation of blood pressure (Petty and Reid, 1981). A role for the peptide substance P in central cardiovascular mechanisms has been suggested by several studies. Substance P is found in abundance within the hypothalmus where this peptide has been observed to exert a potent pressor response (Unger et al, 1981). In contrast, in the medulla this peptide has been observed to produce either a fall in blood pressure, or have no effect (Kubo and Kihara, 1987; Carter and Lightman, 1986). Two other peptides with established influence in cardiovascular regulation are angiotensin II and vasopressin. In addition to the purported centrally-mediated haemodynamic (pressor) activity of circulating systemic angiotensin II and vasopressin, recent studies have identified neuronal systems containing these peptides in the central parenchyma, suggesting an intrinsic central neurotransmitter role for these peptides (Ferrario et al, 1970; De Wied and Jolles, 1982). Although these systems have been observed in a variety of discrete central regions including the brainstem, angiotensin II and vasopressinergic nerves are most prominent in hypothalmic regions. Administration, either into the cerebrospinal fluid or into discrete brain regions have confirmed a pressor response to angiotensin II and vasopressin (Reid and Rubin, 1987). An important role in cardiovascular control mechanisms is also proposed for the peptides

neuropeptide Y and the very recently identified peptide endothelin-1. A function for these peptides in the peripheral cardiovascular system has been described and this together with their imputed central activity will be considered in detail in Sections 1.5 and 1.6.

1.2.3 Cotransmission

It is now apparent that peptides are present in well-defined central neuronal systems and effect the mechanisms involved in cardiovascular control. While these peptides may be present in distinct systems with an independent function, immunohistochemical analysis of the distribution of various peptides has revealed that in many cases both in the peripheral and central nervous systems, peptides could be observed in a neurone containing another transmitter. Several types of co-existing situations have emerged: (1) classical transmitter plus peptide(s); (2) more than one classical transmitter and (3) more than one peptide (Hokfelt et al, 1986). The functional significance of co-existing messengers has not been fully resolved. However, that the various types of compounds differ in parameters such as time course and mechanism of action may be utilised if they are co-stored and/or co-released. Indeed amino acids have been associated with fast transmission processes whereas monoamines and particularly neuropeptides often exert effects which are slow on onset and of long duration (Bloom, 1979; Schmitt, 1984). There are several models which explain how co-stored messengers might work. One mechanism would be that the

neurone always releases all of the messenger molecules simultaneously and the distribution of receptors provides selectivity and specificity (post-synaptic selectivity). These messengers may act independently or interact either at the postsynaptic or presynaptic level. An alternative would be that the neurone has the ability to release the messengers differentially under certain conditions (pre-synaptic selectivity).

Rapid development in the understanding of co-existing messengers has focused on the presence of a classical transmitter together with a peptide. In the peripheral nervous system evidence exists that classical transmitters and peptides are co-released and in addition to an independent action these agents can also interact in a cooperative way on effector cells. For instance, vasoactive intestinal polypeptide is co-stored in the parasympathetic nerves of the salivary gland with acetylcholine and has been shown to enhance the secretory effects of the classical transmitter. In contrast, in the rat vas deferens where noradrenaline and neuropeptide Y are co-stored, the peptide seems to inhibit the release of noradrenaline via a presynaptic action (Lundberg and Hokfelt, 1983; Stjarne et al, 1986). The situation is more confusing in the central nervous system because of the more complex neuronal organisation and the difficulties experienced obtaining suitable preparations for investigation. Although numerous co-existing situations do exist between classical transmitters and peptides in brain regions the

functional significance of these is still rudimentary (Table 1.2). However the function of the co-storage of the classical transmitter serotonin with the peptide substance P in the medullary neurones has been investigated. These neurones project to the spinal cord and probably innervate motoneurones. It has been shown that the peptide blocks the presynaptic serotonin autoreceptor and consequently enhances serotonin release from spinal cord slices. The physiological relevance of this action could be to strengthen the serotonin-mediated stretch reflex (Mitchell and Fleetwood-Walker, 1981). Another system that has attracted considerable attention is the differential co-existence of peptides in central catecholamine neurones. Since adrenaline and noradrenaline neurones have been implicated in cerebral cardiovascular regulation, a role for colocalised peptides in central haemodynamic mechanisms has been suggested. In particular, neuropeptide Y, which is co-stored and interacts with noradrenaline in the peripheral nervous system, has been shown to be present in central catecholamine neurones and may therefore contribute to blood pressure regulation (Section 1.5).

1.3 Importance of the Medulla Oblongata in the Control of the Cardiovascular System

1.3.1 History

Although the potential for control of the cardiovascular system from regions rostral to the brainstem has been acknowledged, historically systems within the lower brainstem, which comprise the medulla oblongata and pons, have been

Table 1.2

Coexistence of Classical Transmitters and Peptides in the Central Nervous System - Selected Examples

Classical	Peptide	Brain region (species)
Dopamine	Neurotensin	Ventral tegmental area (rat)
	Cholecystokinin	Ventral tegmental area (rat, man)
Noradrenaline	Enkephalin	Locus coeruleus (cat)
	Neuropeptide Y	Medulla oblongata (man, rat)
		Locus coeruleus (rat)
Adrenaline	Neurotensin	Medulla oblongata (rat)
	Neuropeptide Y	Medulla oblongata (rat)
	Substance P	Medulla oblongata (rat)
Serotonin	Substance P	Medulla oblongata (rat)
	Enkephalin	Medulla oblongata, pons (rat)
Achetylcholine	Vasoactive intestinal polypeptide	Cortex (rat)
	Enkephalin	Spinal cord (rat)
	Substance P	Pons (rat)
GABA	Somatostatin	Thalmus (rat)
		Cortex (rat)

Data taken from Hokfelt et al (1984a).

recognised as critical for the tonic as well as reflex maintenance of arterial pressure. The first major advance towards this concept was the discovery by Bernard (1863) that transection of the spinal cord resulted in a substantial fall in arterial pressure. Later Dittmar (1873) and Owsjanniko (1871) demonstrated that transection of the brainstem from areas that lie rostral to it had little effect on the basal level of systemic arterial pressure, whereas successive caudal transections produced a progressively greater fall in arterial pressure, until disconnection at the level of the obex reduced arterial pressure to a level comparable to that observed after cervical spinal transection. From these classic studies and the initial failure of investigators to replicate, by localised lesions in the medulla, the effects of spinal cord transection arose the hypothesis that the essential neurones for maintenance of arterial pressure were distributed throughout the brainstem rather than being concentrated in a discrete region. Thus, it was considered that a diffuse pool of neurones were responsible for receiving all the information regarding cardiovascular parameters, transforming it into the provision of sympathetic nerve outflow and consequently regulating systemic arterial pressure. However, with the introduction of the stereotaxic method the brainstem was examined more systematically for sites from which alterations in arterial pressure could be elicited by electrical stimulation. This led to the description of large pressor and depressor areas in the brainstem with a number of groups focusing on the dorsolateral reticular formation of the

brainstem as a potential site of a pool of tonically active neurones. These neurones would provide the excitatory and inhibitory input to sympathetic vasomotor and cardiac nerves (Alexander, 1946; Wang and Ranson, 1939). Around the same time it was realised that the cardiovascular effects elicited by electrical stimulation could result from the excitation of fibres of passage rather than cell bodies. Therefore, the extent of the pressor as well as depressor regions demonstrated previously may reflect the diffuse distribution of axons rather than the 'cardiovascular' cells themselves. This information suggested that the central neurones controlling sympathetic cardiac and vasomotor outflow were perhaps situated in a discrete region(s).

The ventral surface of the medulla was next to come under scrutiny as a site of vasomotor activity. This was initially because of the observations of Guertzenstein (1973), that chemical inhibition, in particular direct application of sodium pentobarbitone or glycine resulted in a profound fall in arterial pressure similar to spinal cord transection. Since glycine was known to hyperpolarise and inactivate neurones by an action on the cell bodies rather than axons, it was postulated that the neuronal cell bodies near the ventral surface provided the tonic excitatory input to the sympathetic preganglionic nerves. These neurones regulate the activity of the neurones innervating the heart and blood vessels. The availability of neuroanatomical techniques such as the use of fluorescent dyes and markers (horseradish peroxidase) that provide tracings of pathways

greatly advanced the knowledge of neuronal connections within the central nervous system. Using the latter technique Amendt et al (1979) confirmed that a group of neurones located near the ventral surface of the medulla projected directly to the intermediolateral cell columns of the spinal cord where the preganglionic neurones, which regulate the activity of the nerves to blood vessels and the heart, are situated.

Altogether the evidence suggested that a group of neurones in the ventrolateral medulla were critical for the maintenance of vasomotor tone and strengthened the hypothesis that vasomotor activity is regulated by functionally discrete medullary neurones rather than a general multifunctional neuronal pool. While this area is ostensibly of crucial importance in sustaining arterial blood pressure, other major areas in the medulla have since been observed to have important functions per se in the tonic and reflex control of blood pressure in addition to regulating the tonically active neurones in the ventrolateral medulla. The following sections will discuss in more detail the neuroanatomy, physiology and transmitters of these major medullary nuclei (incorporating the ventrolateral regions) whose function and interconnections are considered pivotal to the regulation of the cardiovascular system.

1.3.2 The Nucleus of the Solitary Tract

The nucleus of the solitary tract (NTS), situated in the dorsomedial medulla is the main terminus and relay station for cardiovascular afferent information (as well as other visceral

information) concerning the status of the peripheral cardiovascular function (Figure 1.3). Thus, baroreceptors located in the heart, carotid sinus, aortic arch and other large vessels, detect changes in blood pressure and transmit this information via fibres which travel in the carotid sinus nerve, as well as the glossopharyngeal and vagus nerves to the NTS. This innervation is bilateral and the fibres terminate in the caudal (dorsolateral, medial, commissural) two-thirds of the logitudinal Y-shaped nucleus (Calaresu et al, 1975; Wallach and Loewy, 1980).

The major role of the NTS is considered to be in the mediation of the baroreceptor reflex. This cardiovascular reflex is stimulated by increases in arterial blood pressure and evokes both a decrease in peripheral vascular resistance as well as a negative chronotropic and inotropic effect on the heart. The involvement of the NTS in the reflex regulation of cardiovascular function is supported by the finding that neurones in the NTS respond monosynaptically to stimulation of baroreceptor afferents and fire in synchrony with the arterial pulse, indicating that they are locked into the baroreceptor reflex chain (Spyer, 1981). Moreover, Doba and Reis (1973) demonstrated the lesioning of this nucleus abolished baroreflexes and resulted in an acute elevation of blood pressure (neurogenic hypertension), whereas electrical stimulation of this nucleus elicits the opposite response with a reduction in blood pressure (Seller and Illert, 1969). Although controversial, the rise in arterial pressure with discrete lesions of the NTS has been reported by several groups to return

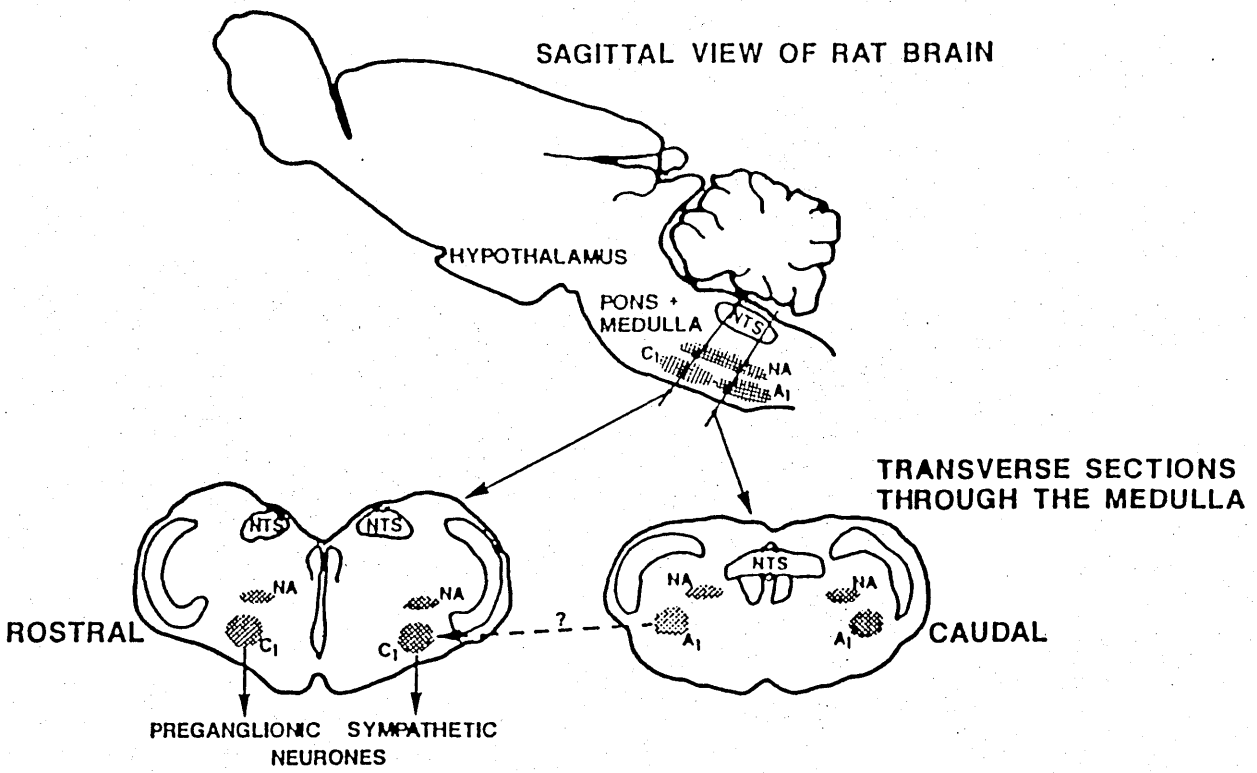


Figure 1.3

Schematic diagram showing the location of catecholamine cell groups in the medulla involved in the control of blood pressure and heart rate.

NTS, Nucleus of the solitary tract; NA, nucleus ambiguus, A_1 noradrenergic cell group, C_1 adrenergic cell group.

to normal values with time, suggesting that this nucleus is not crucial for establishing the long-term set point for blood pressure. However, a permanent increase in blood pressure lability has been demonstrated (Cowley et al, 1973; Bucholz and Nathan, 1984). A prominent feature of the NTS is the number of putative neurotransmitter substances that have been localised and that produce a haemodynamic response in this region (Malay and Elde, 1982; Yamazoe et al, 1984; Leslie, 1985). The transmitter of the primary afferent input is uncertain. Substantial evidence exists to suggest that afferent fibres may contain the excitatory amino acid L-glutamate and that release of this amino acid transmits the information from the baroreceptors onto the neurones of the NTS. Indeed, local injection of L-glutamate into the NTS elicits baroreflex-like responses (Reis et al, 1981). That substance P is also present in these afferent fibres implicates this peptide as a possible transmitter in the baroreflex. However its function in this region is less clear since it has been shown to either decrease blood pressure or have no effect (Gillis et al, 1980; Kubo and Kihara, 1987; Carter and Lightman, 1986). Catecholaminergic neurones and terminals have also been located in the NTS. This region contains two notable catecholamine cell groups, the noradrenaline A_2 cell group located in the caudal region and the more rostral group of adrenergic C_2 neurones (Section 1.2.1). Morover, several studies have shown that catecholamines can function as neurotransmitters in the NTS to regulate autonomic function (Zandberg et al, 1979).

Although the NTS does not have extensive projections to the preganglionic sympathetic neurones which control vasomotor outflow, it does project to several brainstem and forebrain sites that do have direct sympathetic and vagal connections (Loewy and Burton, 1978). Indeed the modulation of arterial pressure via brainstem nuclei which control preganglionic nerve activity is considered to be of critical importance in the regulation of arterial pressure in the NTS-mediated baroreflex response. Electrophysiological and neuroanatomical studies provide evidence of a reciprocal connection between the NTS and the ventrolateral medulla, supporting such an involvement (Ciriello and Caverson, 1986; Dampney et al, 1987; Ruggiero et al, 1985b). Furthermore, lesions of the ventrolateral region, in particular the rostral portion, abolish baroreceptor and other reflex responses elicited from afferent fibres of the vagus (Section 1.3.4). The NTS also has neural connections with higher structures in the brain. The parvocellular neurons in the paraventricular nucleus of the hypothalamus receive an input from the A2 cell group of the NTS. Projects from the parvocellular neurones to the autonomic regions of the brainstem and spinal cord are thought to contribute to the control of cardiovascular function (Sawchenko and Swanson, 1982; Ciriello and Calaresu, 1980b; Brody, 1986). It has been demonstrated that the NTS can modulate the electrical activity of the magnocellular neurones in the hypothalamic supraoptic and paraventricular nuclei and thus regulate secretion of the peptide vasopressin from the neurohypophysis (Koizumi and Yamashita,

1978; Harris, 1979; Shade and Share, 1975). Pathways from the NTS via the parabrachial nucleus or the A_1 cell group of the caudal ventrolateral medulla have been implicated in the provision of the ascending pathways controlling the secretion of this vasoconstrictor and antidiuretic hormone (Norgren, 1978; Swachenko and Swanson, 1982). The integrative function of the NTS in cardiovascular regulation is further highlighted by the diversity of central sites which have inter connections with, and are able to modulate the function of, this nucleus. These include inputs from the area postrema, parabrachial nucleus of the pons, the fastigial nucleus, and the hypothalamus (Ross et al, 1981b). A pathway from the NTS to the nucleus ambiguus of the medulla is thought to contribute to the reflex regulation of heart rate (Steusse and Fish, 1984a). The involvement of the latter nucleus in cardioinhibitory function will be considered in the following section.

1.3.3 The Nucleus Ambiguus

The nucleus ambiguus (NA) has been proposed as a site of origin of central cardioinhibitory neurones (Figure 1.3). This nucleus consists of a thin column of cells in the ventrolateral medulla, extending from the caudal pole of the facial nucleus to the pyramidal descussation. The first suggestion that this nucleus was involved in cardioinhibitory functions was based on the finding that lesions of the nucleus produced degeneration in the cardiac branches of the vagus (Szentagothai, 1952). More recent histochemical studies in the rat have confirmed that the

nucleus ambiguus sends projections through the vagus to the heart and that the majority of preganglionic parasympathetic fibres which terminate in the heart originate from the NA (Stuesse, 1982). Another region in the medulla, the dorsal motor nucleus of the vagus is also known to send projections through the vagus (Kalia, 1981). However, electrophysiologic functions have failed to clarify the role the projections from this nucleus play in cardioinhibition. Electrical stimulation has been reported to either result in bradycardia or have no effect (Geis and Wurster, 1980; Steusse and Fish, 1984b). While species' differences may account for these discrepancies, recent evidence suggests that this area is predominately concerned with altering heart contractility but not heart rate (Geis et al, 1981).

Thus, consistent with its purported role as a cardioinhibitory centre electrical stimulation of the NA results in bradycardia (Ciriello and Calaresu, 1980c). The area of the NA which reduces the heart rate has been reported to be confined to a small rostral portion of the nucleus (Steusse and Fish, 1984a). These authors further demonstrated by tracer techniques that a major projection to this rostral area was from the NTS, a known site of termination of cardiovascular afferent fibres. Indeed selective lesions of the NTS were previously shown to result in the degeneration of projections to the NA implicating the latter nucleus in the pathway mediating reflex cardiac control. Moreover, Machado and Brody (1988a; 1988b) have proposed that this area also has a tonic cardioinhibitory function. Electrical stimulation of the NA evoked a bradycardia

that was not reflex in origin since it was maintained in rats with sinoaortic deafferation. Other projections to this region were derived from the parabrachial complex and the paraventricular nucleus of the hypothalamus (Steusse and Fish, 1984a). Although these areas might be involved in the slowing of heart rate, other autonomic functions may also be subserved. The view that the NA may be related to the control not only of heart rate but also of arterial pressure was purported by Machado and Brody (1988a; 1988b). Since electrolytic, as well as chemical lesion, facilitated the development of hypertension in rats with sinoaortic deafferation. In this respect the functional role of the established projections from the NA to the NTS, the parabrachial nucleus and the spinal cord have to be further investigated. Another potentially important functional connection is that reported from the NA to the rostral ventrolateral medulla (Dampney et al, 1987). The role of this input from the NA to this area is as yet unknown. However, the importance of the rostral ventrolateral medulla in the regulation of blood pressure, which will be discussed in the proceeding section, implicates the NA in more widespread functions than cardioinhibition.

1.3.4 The Rostral Ventrolateral Medulla

Following the initial observation that glycine applied to the ventral surface of the medulla caused a collapse of arterial pressure comparable to spinal cord transection, this general area of the medulla has been subjected to extensive investigations as

a possible site of vasomotor activity (Guertzenstein, 1973). Several groups have since demonstrated that electrical or chemical lesioning of a discrete region in the rostral ventrolateral medulla (RVLM) reduced arterial pressure and heart rate to spinal levels. This evidence together with the fact that electrical or chemical excitation of this region elevates arterial pressure and heart rate, suggests that the neurones of this restricted zone are sympathoexcitatory and that their integrity is necessary for the maintenance of vasomotor tone (Dampney et al, 1982; Ross et al, 1984b).

In a series of experiments combining electrophysiological as well as immunohistochemical techniques, Ross et al (1984b) revealed a tight correlation in the rat between the location of the RVLM pressor region and a group of adrenaline-containing cell bodies, originally designated the C_1 cell group (Hokfelt et al, 1974; Section 1.2.1). The C_1 area of the RVLM in the rat is located caudal to the facial nucleus of the pons and rostral to the most precerebellar relay neurones of the lateral reticular nucleus (Figure 1.3). It extends rostrocaudally as a cell column, representing a subdivision of the nucleus paragigantocellularis lateralis (Paxinos and Watson, 1986; Andrezik et al, 1981). Anatomical studies have shown that this adrenaline-synthesising cell group projects to the intermediolateral cell column of the spinal cord, where the preganglionic neurones are situated, thus supporting a functional role for the intrinsic neurones in the C_1 area in tonic vasomotor

tone (Ross et al, 1984a). Indeed the changes in arterial pressure subsequent to pharmacological or electrophysiological manipulation of the RVLM C_1 region are associated with the alteration of sympathetic nerve activity and differential regulation of vascular resistance (Ross et al, 1984b; Willette et al, 1987). Whether or not adrenaline is the transmitter that is released to excite the preganglionic neurones in the spinal cord remains to be established. That the microiontophoresis of adrenaline in the area of the preganglionic neurones apparently inhibits rather than exites the discharge of sympathetic nerve activity is a perplexing paradox (Guyenet and Cabot, 1981). It is conceivable that microinotophoretically applied adrenaline may act on a different population of receptors than those which are innervated by the preganglionic sympathetic neurones. Moreover, the discovery of coexisting peptides such as neuropeptide Y or substance P in the C_1 neurones raises the possibility that the release of some agent other than adrenaline produces sympathoexcitation (Hokfelt et al, 1983a; Lorenz et al, 1985). Pilowsky et al (1987) have demonstrated that chemical stimulation of C_1 neurones results in a pressor response and release of NPY-like immunoreactivity from the rabbit spinal cord. The concept of co-existing transmitters in relation to the catecholamine neurones of the ventrolateral medulla will be discussed in Section 1.5. However, the controversy surrounding the transmitter released from the C_1 neurones onto the preganglionic neurones does not detract from the ostensible importance of these neurones in tonic vasomotor control. It has been suggested that

ultimate tonic control over the sympathetic nerves is synchronised by central mechanisms that generate the periodicity of sympathetic activity (Gebber, 1984). On this basis two regions, including the RVLM, have been proposed as mediating basal vasomotor tone. Electrophysiological studies have demonstrated that neurones in the dorsomedial medulla whose activity is time locked to the slow wave of sympathetic nerve activity, discharge earlier than neurones in the RVLM. This implies that although both regions participate in the generation of spontaneous vasomotor outflow, the former area may predominate. However, since neurones in the RVLM project to the intermediolateral cord whereas the dorsomedial neurones do not, it has been postulated that the fall in arterial pressure produced by interruption of the dorsal medial site results from the blockade of RVLM fibres that project through this region before descending to the spinal cord (Dampney et al, 1982). Thus, the RVLM has been proposed as the area providing the tonic excitatory input to the spinal sympathetic preganglionic neurones, with the dorsal medulla a part of the pathway mediating basal vasomotor tone.

RVLM participation in reflex as well as tonic control of the cardiovascular system (Section 1.3.2) is suggested by the existence of a reciprocal connection of this area with the NTS, upon which afferent fibres mediating baro- and other cardiopulmonary reflexes terminate (Dampney et al, 1987; Ruggiero et al, 1985b; Ciriello and Caverson, 1986). The C_1 neurones of

this area are again implicated since projections from the cardiovascular portions of the NTS directly overlap with the region of the RVLM containing the C_1 neurones. Moreover the vasodepressor response elicited by stimulation of the vagus nerve or carotid sinus stretch is abolished following bilateral chemical blockade of the C_1 area (Granata et al, 1983). Conversely, withdrawal of the baroreceptor input by lesion of the NTS results in an elevation of arterial pressure which can be abolished by interference with the C_1 neurones (Benarroch et al, 1986). Thus the involvement appears to be that of an inhibitory pathway from the NTS to the C_1 area which can reduce the spontaneous drive imposed by the C_1 cell group on the preganglionic sympathetic neurones. As discussed recently by Reis and colleagues (1987; 1988) the C_1 area is under the control of a variety of proposed neurotransmitters. For instance, acetylcholine and glutamate are associated with an excitatory input to the C_1 neurones whereas noradrenaline and gamma-aminobutyric acid (GABA) are inhibitory. GABA may be the inhibitory transmitter in the NTS-C_1 pathway, but it is more likely to be present in local interneurones (Meeley et al, 1985; Ruggiero et al, 1985b). Neurones in the RVLM, in particular the C_1 neurones, may mediate another important reflex response. Dampney and Moon (1980) reported that the potent reflex response to cerebral ischemia is abolished by bilateral lesions of this region, a subject that will be discussed in more detail in Section 7.3.

The number of convergent inputs into the RVLM further support its integrative function in the cardiovascular system. Neuroanatomical studies have described connections from the parabrachial and Kolliker-Fuse nuclei of the pons, the nucleus ambiguus as well as the paraventricular and lateral hypothalamic regions (Dampney et al, 1987). While the exact function of some of these descending pathways have not been fully defined, selective inhibition of C_1 neurones attenuates the vasoconstrictor response that can be elicited from the hypothalamus. This pathway may therefore be another potential route by which the parvocellular neurones of the paraventricular nucleus can influence autonomic function, in addition to its direct inputs to the spinal cord and dorsomedial medulla (Hilton et al, 1983; Swanson and Kupers, 1980).

The caudal ventrolateral medulla with connections to the hypothalamus reputedly influences the C_1 area. This vasodepressor region is juxtapositioned to the C_1 area on the rostrocaudal plane and its involvement with the C_1 region and haemodynamic function per se will be discussed in the next section.

1.3.5 The Caudal Ventrolateral Medulla

The caudal ventrolateral medulla (CVLM) at its most rostral extent overlaps with the pressor neurones of the RVLM (Figure 1.3, Paxinos and Watson, 1986). In contrast to the RVLM, electrical or chemical stimulation of the CVLM results in an acute fall in arterial pressure and heart rate (Blessing and

Reis, 1982; Willette et al, 1987). A tonic inhibition of vasomotor activity by this region has been proposed, since electrical or chemical inhibition elicits a marked increase in arterial pressure (Blessing et al, 1982). The cardiovascular responses produced by such interventions are reputedly mediated through the A_1 noradrenergic neurones contained within the CVLM (Dahlstrom and Fuxe, 1964, Section 1.2.1). Support for this hypothesis derives from anatomical correlations, the manipulations being deliberately made in the region coinciding with the A_1 cell group. Although this association has been questioned recently by Day et al (1983), functional studies as well as neuroanatomical investigations of the efferent connections of the CVLM, have focused on the A_1 cell group. The pathway through which the vasodepressor area alters arterial pressure has not been established with certainty, however most of the recent evidence indicates that few, if any, of these noradrenergic cells project to the spinal cord (Ross et al, 1981a; Blessing et al, 1981a). Although non-catecholamine neurones in the CVLM are known to project to the spinal cord, it is generally considered that the sympathoinhibition exerted by activation of the A_1 neurones is relayed via other structures. The principal efferent connections from the A_1 neurones that have been anatomically established include projections to the paraventricular and supraoptic nuclei of the hypothalamus as well as the NTS (Sawchencho and Swanson, 1982; Blessing et al, 1981b). Since neurones from the parvocellular component of the

paraventricular nucleus project directly and indirectly to the spinal cord to influence sympathetic nerve activity, the A_1 neurones could potentially regulate cardiovascular function through these areas. However, close proximity of A_1 region to the RVLM, has tempted some investigators to suggest an inhibitory input from A_1 to the C_1 cell group. Pharmacological evidence for such a mechanism has been accrued recently. The hypertensive response evoked by microinjection of muscimol or kainic acid into the CVLM was abolished by chemical inhibition of the RVLM (Willette et al, 1984; Granata et al, 1986). In contrast, the latter study also demonstrated that the hypertensive response was not altered by interruption of the two other major projections of the A_1, namely to the hypothalamus or to the NTS. Moreover, hypotension evoked by CVLM stimulation in the rat, which should according to the hypothesis release noradrenaline onto the C_1 neurones is mimicked by local application into the C_1 area of the α-agonist alpha-methyl noradrenaline (Granata et al, 1986).

Although the C_1 area contains noradrenaline synthesising nerve terminals, the neuroanatomical evidence that these derive from A_1 projections in nebulous. Chan et al (1986) attest, on the basis of horseradish peroxidase tracer techniques in the rat, that a direct projection exists from the CVLM to the RVLM whereas in the rabbit, Blessing et al (1987a) maintain that neither a direct nor indirect (via other noradrenergic pathways in the brainstem) noradrenergic projections exist between these medullary areas. Species variation may account for these discrepant reports. However it is possible that a non-

noradrenergic inhibitory projection from the CVLM to the RVLM may exist and contribute to the functional interaction between the regions. Indeed Urbanski and Sapru (1988) have pharmacological evidence for a GABAergic pathway between the CVLM and RVLM. Another potential mechanism by which the A_1 region could influence the C_1 area and cardiovascular function is by the release of co-existing transmitters in this area. In this respect the putative role of the colocalised neuropeptide Y is of particular interest.

Sawchenko and Swanson (1982) have demonstrated an ascending projection from the A_1 neurones to the magnocellular division of supraoptic and paraventricular nuclei, where vasopressinergic cell bodies are concentrated. Thus it was proposed that the A_1 neurones may regulate the release of vasopressin (AVP), a potent vasoconstrictor and antidiuretic hormone, with a homeostatic function in situations (haemorrhage) where blood volume is threatened (Johnston, 1985).

Blessing et al (1982) originally postulated that A_1 neurones tonically inhibit AVP release since electrolytic lesioning of this area resulted in an increase in AVP. However, controversy surrounds the contribution that this elevation in AVP made to the accompanying hypertension. Although Blessing et al (1982) suggested that the rise in AVP was responsible for the effect on blood pressure, Minson and Chalmers (1983) have proposed that AVP makes a greater contribution to the bradycardia than to the hypertension after A_1 lesions. More specific studies by Blessing

and Willoughby (1985) have since demonstrated that chemical inhibition of the CVLM prevents the release of AVP induced by haemorrhage whereas chemical stimulation elevates the plasma AVP levels. These authors thus concluded that contrary to the original hypothesis by Blessing et al (1982), the A_1 noradrenergic pathway to the hypothalamus facilitate the secretion of AVP. While the significance of the A_1 neurones in reflex (haemorrahagic) regulation of AVP release and heart rate have been confirmed, the involvement of these neurones in the reflex control of blood pressure is controversial (Head et al, 1987a). Lesions of the A_1 area, in contrast to the C_1 area, do not substantially alter the magnitude of the vasodepressor response to stimulation of vagal afferent fibres or to carotid sinus stretch, implying that the caudal region does not affect reflex arterial pressure. However, participation in the vasodepressor response elicited after aortic nerve stimulation has been reported suggesting that the central mechanisms mediating reflex blood pressure control differ depending on the afferent input (Granata et al, 1985; Gordon, 1987).

1.4 Second Messenger Systems Mediating Neurotransmitter or Hormonal Actions

Neurotransmitters and hormones act upon specific recognition sites located on cell membranes and it is considered that activation of these sites modifies cellular activity and can potentially elicit a vast array of physiological responses. The initial stage in this process, transmitter recognition, is

translated into altered cellular function through a coupling between the recognition site and an effector mechanism. In some cases, the recognition site is directly associated with an ion channel, whereas in others the effector is a membrane-associated enzyme that generates the production of a second messenger. There are two major receptor-regulated second messengers currently thought to be present in the brain; the adenylate cyclase system and the phosphoinositide cycle. The mechanisms whereby these systems modulate synaptic transmission following receptor activation will be outlined below. Special emphasis will be given to the adenylate cyclase system since studies within this thesis examine the role of this system in central peptidergic cardiovascular regulatory mechanisms.

1.4.1 Adenylate Cyclase System

The more established of the two second messenger systems is associated with the enzyme adenylate cyclase and involves the generation of cyclic adenosine 3'5-monophosphate (cAMP) from adenosine triphosphate (ATP) as depicted in Figure 1.4. cAMP then acts as an intracellular second messenger to activate protein kinases and subsequently modify cellular activity. A role for cAMP in mediating cellular events has been known since the late 1950s when Sutherland and coworkers suggested it was a cofactor enabling adrenaline to initiate glycogen breakdown in the liver (Sutherland and Rall, 1960). A feature common to both the adenylate cyclase system and the phosphoinositide cycle is the involvement of guanine nucleotide binding proteins (G-

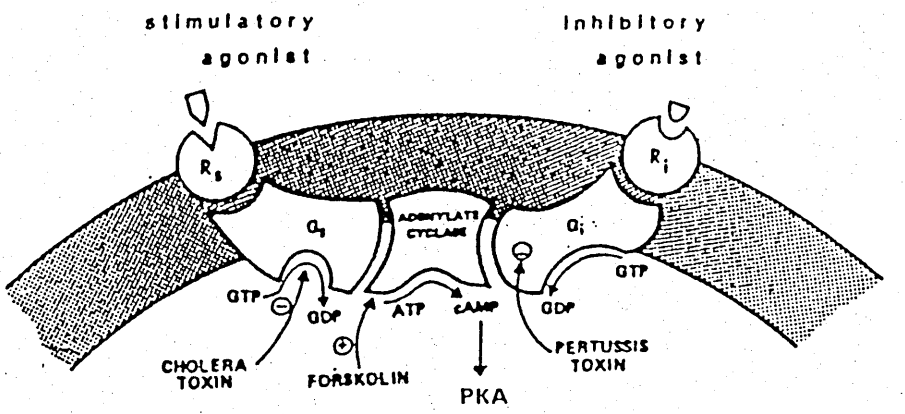


Figure 1.4

Dual regulation of adenylate cyclase

Rs = stimulatory receptor

Ri = inhibitory G-protein

Gs = stimulatory receptor

Gi = inhibitory G-protein

GTP = guanosine triphosphate

GDP = guanosine diphosphate

ATP = adenosine triphosphate

AMP = adenosine monophosphate

See text for details of this system

proteins). G-proteins, which consist of three subunits, α , β and γ , transduce and amplify the extracellular signal into enzymatic activity. Receptors that stimulate adenylate cyclase, such as β-adrenergic and A_2-adenosine receptors, do so by activation of a specific G-protein (Gs), (Figure 1.4). Prior to receptor activation Gs is complexed with guanosine diphosphate (GDP) and is unable to stimulate adenylate cyclase. Receptor activation of GS enhances the affinity of the α subunit for ganosine triphophate (GTP) relative to GDP. In turn, the activated α subunit-GTP dissociates from the $\beta\gamma$ subunit and stimulates adenylate cyclase activity, which then catalyses the conversion of ATP to cAMP. Termination of the transmitter signal involves hydrolysis of GTP to GDP by GTPase activity of the α-subunit and possibly by reassociation of the subunits. The continued presence of a hormone at the receptor permits the repetition of this cycle of events (Levitzki, 1987).

Another distinct G protein (Gi) is involved in the inhibition of adenylate cyclase by receptors such as angiotensin II, dopamine (D_2), opiate (μ) and α_2-adrenoceptors (Figure 1.4). The mechanisms involved in the hormonal inhibition of adenylate cyclase are not as well understood as hormonal stimulation. Activated Gi was initially thought to inhibit adenylate cyclase activity directly and thus reduce cAMP production. However, unlike the stimulatory branch, the α subunit of Gi does not form a strong complex with adenylate cyclase. This led to the suggestion the the $\beta\gamma$ -subunit of Gi plays an important role in the inhibitory regulation of adenylate

cyclase possibly by indirectly reducing the activity of Gs. The exact roles of the $\beta\gamma$ subunits in this mechanism, however, remain to be elucidated (Gilman, 1984; Levitzki, 1987).

Thus, according to the receptor type occupied, the production of cAMP will either increase or decrease. The consequences of this modulation of cAMP levels are diverse. This cyclic nucleotide can activate phosphorylating enzyme(s) (protein kinase A) which regulate a variety of cellular responses including metabolic, contractile and secretory events. In a number of these situations cAMP acts to regulate another second messenger, namely calcium ions. Modulation of the calcium pathway by the cAMP system has emerged in cardiac and other muscle cells with a resultant alteration in contractile events. Ion channels in neuronal membranes are also key targets of cAMP action, with secretion of transmitters and neuronal activity being affected. The cAMP-mediated control of calcium and potassium conductance through ion channels involved in neuronal secretory processes, can either facilitate or suppress transmitter release depending on the receptor type activated and the system studied (Illes, 1986). Neuronal activity of the locus coeruleus cells is affected by the regulation of one type of potassium channel by cAMP. A reduction in cAMP levels facilitates the opening of potassium channels causing hyperpolarisation and depression of neuronal firing (Andrade et al, 1985).

Useful tools in the study of the cyclic nucleotide system

include agents which can interact with the different G-proteins. Pertussis toxin blocks the inhibitory action of Gi whereas cholera toxin blocks the GTPase activity of Gs causing its persistent activation (Figure 1.4). The diterpene forskolin, which at high concentrations directly activates adenylate cyclase thereby increasing cAMP, is useful in the examination and detection of inhibitory influences on adenylate cyclase activity. Phophodiesterase inhibitors such as theophylline are effective in studies involving the measurement of cAMP levels since they prevent the breakdown of cAMP to 5'-adenosine monophosphate.

1.4.2 Phosphoinositide Cycle

The more recently discovered second messenger system involved in neurotransmitter and hormonal actions, the phosphoinositide cycle, has been reviewed recently by Berridge and Irvine (1989) and will be discussed briefly. Currently accepted views purport that the neurotransmitter interaction with receptors activates a phosphodiesterase (phospholipase C) that specifically hydrolyses the membrane phospholipid, phosphotidylinositol 4, 5-bisphosphate (PIP_2) (Figure 1.5). Hydrolysis of PIP_2 produces at least two second messengers - diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). This process is analogous to the receptor stimulation of adenylate cyclase, in that transmitters act at receptors, a G protein transduces this extracellular signal activates an enzyme that generates second messengers (Cockcroft and Gomperts, 1985). The involvement of Gi-like proteins has been demonstrated in non-

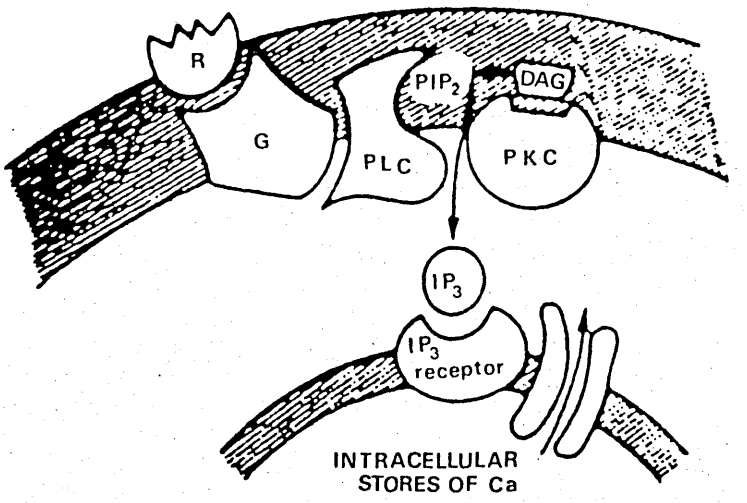


Figure 1.5

Receptor mediated phosphoinositide turnover

R	=	receptor
G	=	G-protein
PLC	=	phospholipase C
PKC	=	protein kinase C
DAG	=	diacylglycerol
PIP_2	=	phosphotidylinositol 4,5-biphosphate
IP_3	=	inositol 1,4,5-triphosphate

See text for details of this system.

neuronal systems, where phosphoinositide turnover is blocked by pertussis toxin or by GTP analogs that inactivate G-proteins. Although several G-proteins have been identified in the brain including Gs, Gi, Go and Gp, the G-protein(s) that mediate stimulation of the phosphoinositide system in the brain remains to be clarified.

Transmitters known to stimulate the phosphoinositide system in the central nervous system include serotonin ($5\text{-}HT_2$ receptors), noradrenaline (α_1-adrenoceptors) and vasopressin (V_1). The messengers generated by phosphoinositide turnover, IP_3 and DAG, modulate a plethora of cellular processes including metabolism, secretion, contraction, neural activity and cell proliferation. The messenger functions of IP_3 involve the interacting with a specific receptor causing the release of calcium from nonmitochondrial, internal stores. The IP_3 signal is terminated by the cleavage of the 5-phosphate, generating inositol 1,4-bisphosphate which is less active (Berridge and Irvine, 1989). In nonneural tissues the action of IP_3 mediates contractile activity. The mechanisms of pharmacomechanical-coupling in smooth muscle and excitation-contraction coupling in skeletal muscle both appear to involve phosphoinositide turnover. At present, the role of IP_3 in the central nervous system has not been fully established, however a participation in neuronal activity and perhaps secretory mechanisms have been suggested. Diacylglycerol, the other major second messenger generated by hydrolysis of PIP_2 activates a phosphorylating enzyme(s) known as protein kinase C which in turn phosphorylates several cellular

proteins (Figure 1.5). The activation of protein kinase C is complex and apparently requires calcium and phospholipid for activity (Nishizuka, 1988). Protein kinase C can act synergistically with calcium pathways to affect a range of physiological responses. A negative feedback regulation of agonist-induced IP_3 production has also been reported for this enzyme. Important functions of protein kinase C include neurotransmitter release, smooth muscle contraction, gene expression and cell proliferation. Protein kinase C also affects neuronal activity. In the hippocampus, activation of this enzyme blocks a calcium-activated potassium channel resulting in enhanced neuronal excitation (Baraban et al, 1985).

In conclusion, there are two major second messenger systems, the adenylate cyclase system and the phosphoinositide cycle, both of which have been identified in the brain and are considered to play a role in the mediation of neurotransmitter function. The participation of the former system in the central haemodynamic effects of neuropeptide Y will be investigated during the course of this thesis.

1.5 Neuropeptide Y

1.5.1 Isolation and Identification of Neuropeptide Y

Neuropeptide Y (NPY), was originally isolated from the porcine brain by Tatemoto et al (1982) and subsequently shown by Tatemoto (1982) to exhibit a sequence of 36 amino acids with an N-terminal tyrosine (Y) and a C-terminal tyrosine amide residue.

This peptide belongs to the biologically active pancreatic peptide (PP) family and as such displays considerable C-terminal amino acid sequence homology, as depicted in Table 1.3, to both bovine PP (17 amino acids in common) and avian PP (20 amino acids in common) as well as to the other cognate peptide, peptide YY (Tatemoto et al, 1982). Investigation of the molecular origins of this peptide has resulted in the determination of the structure and expression of the NPY gene in the rat. The predicted amino acid sequence of mature rat NPY is identical to the human sequence (Larhammar et al, 1987). No precursor has yet been isolated from tissue extracts, however the structure of the human NPY precursor has been deduced from cloning experiments to be a 97 amino acid residue. This putative precursor consists of three peptide segments, a leader sequence of 28 amino acids, an active neuropeptide Y sequence of 36 amino acids and a 30 amino acid carboxyterminal peptide (Minth et al, 1984).

Although NPY was first described in the porcine brain, immunohistochemical as well as radioimmunoassay studies using antibodies raised towards this peptide, have revealed a pervasive distribution of NPY-like peptide in both the peripheral and central nervous systems. This wide but discrete distribution of NPY resembles that of the previously mapped pancreatic polypeptides (Lundberg et al, 1984; Allen et al, 1983a). While radioimmunoassay techniques confirmed the presence of NPY, this analysis could not detect PP-like immunoreactivity. Indeed it has now been established that cross-reactivity to NPY of the avian-PP and bovine-PP directed antisera had occurred in the

Table 1.3

Amino Acid Sequences of Neuropeptide Y and Three Cognate Peptides

Amino acid sequence	NPY	APP	BPP	PYY
1	Tyr	Gly	Ala	=
2	Pro	=	=	=
3	Ser	=	Leu	Ala
4	Lys	Gln	Glu	=
5	Pro	=	=	=
6	Asp	Thr	Glu	Glu
7	Asn	Tyr	Tyr	Ala
8	Pro	=	=	=
9	Gly	=	=	=
10	Glu	Asp	Asp	=
11	Asp	=	Ala	=
12	Ala	=	=	=
13	Pro	=	Thr	Ser
14	Ala	Val	Pro	Pro
15	Glu	=	=	=
16	Asp	=	Gln	Glu
17	Leu	=	Met	=
18	Ala	Ile	=	Ser
19	Arg	=	Gln	=
20	Tyr	Phe	=	=
21	Tyr	=	Ala	=
22	Ser	Asp	Ala	Ala
23	Ala	Asn	Glu	Ser
24	Leu	=	=	=
25	Arg	Gln	=	=
26	His	Gln	Arg	=
27	Tyr	=	=	=
28	Ile	Leu	=	Leu
29	Asn	=	=	=
30	Leu	Val	Met	=
31	Ile	Val	Leu	Val
32	Thr	=	=	=
33	Arg	=	=	=
34	Gln	His	Pro	=
35	Arg	=	=	=
36	Tyr*	=	=	=

Ala - Alanine; Arg - Arginine; Asn - Asparginine; Asp - Aspartic acid; Glu - Glutamic acid; Gln - Glutamine; Gly - Glycine; His - Histidine; Ile - Isoleucine; Leu - Leucine; Lys - Lysine; Meth - Methionine; Phe - Phenylalanine; Pro - Proline; Ser - Serine; Thr - Threonine; Tyr - Throsine; Val - Valine.

* - Amidation of Y terminal; = Identical amino acid to NPY

From Tatemoto et al (1982) and Tatemoto (1982).

previous investigations and that the preponderance of PP-like immunoreactivity hitherto observed, was actually due to NPY (Lundberg et al, 1984; DiMaggio et al, 1985; Allen et al, 1983a). That NPY is the predominant pancreatic peptide in the central nervous system is further supported by evidence that the other structurally related peptide, peptide YY is largely confined to the gut (Lundberg et al, 1984).

1.5.2 Distribution of Neuropeptide Y in the Peripheral Nervous System

In the peripheral nervous system, high levels of NPY (up to 80 pmol g^{-1}) have been identified in the sympathetic ganglia and the heart with significant levels in other tissues such as the spleen, bladder and respiratory system. This peptide has been localised immunohistochemically in the perivascular nerves innervating the blood vessels of the heart, kidney, liver, spleen, skeletal muscle and in the gastrointestinal tract with the concentration in each area generally reflecting the density of innervation for that organ. These findings have been reported in a number of species including rat and man (Gu et al, 1983; Lundberg et al, 1983; 1984; Ekblad et al, 1984; Uddman et al, 1985).

A salient feature of the location of NPY in the periphery is its occurrence predominately with noradrenaline in sympathetic ganglia and sympathetic neurones in many tissues. Immunohistochemical studies have also shown that colocalisation of this peptide and noradrenaline is a hallmark of a certain

population of sympathetic neurones, namely those that innervate the cardiovascular system (Lundberg et al, 1983; Ekblad et al, 1984; McLachlan and Llewellyn-Smith, 1986). In addition, NPY has been closely associated or colocalised with noradrenaline in chromaffin cells of the adrenal medulla reportedly in concentrations greater than in the rat brain (Allen et al, 1983a; 1983b). At the ultrastructural level, it has been observed that noradrenaline and NPY may be colocalised not only within the same nerve cells or fibres but within the same vesicle, namely in large dense-cored vesicles. Most noradrenaline however, is stored in small dense-cored vesicles which do not contain NPY (Fried et al, 1985a; 1985b). A functional significance of these histochemical observations is implied by the finding that these messengers can be differentially released. Continuous low frequency stimulation has been shown to release noradrenaline preferentially from small vesicles in the spleen where as high frequency stimulation releases both noradrenaline and NPY from large vesicles (Lundberg et al, 1986). NPY has also been localised in non-vascular neurones which do not contain noradrenaline, for instance, in the heart and in the gastrointestinal tract (Hassal and Burnstock, 1984; Sundler et al, 1983).

1.5.3 Distribution of Neuropeptide Y in the Central Nervous System

The presence of NPY-like immunoreactive material has been observed throughout the rostrocaudal extent of the mammalian

(including rat, cat and human) neuroaxis (Allen et al, 1983a; Chronwall et al, 1985; Gibson et al, 1984; Adrian et al, 1983). NPY is present in amounts which exceed (in picomole/gram of tissue quantities) those of other known peptides in the mammalian central nervous system including cholecystokinin (Allen et al, 1983a). Although reports differ regarding the absolute values of NPY in each of the central areas, the concentration of peptide measured by radioimmunoassay is not uniform between regions and may also be dependent on the species examined. As demonstrated for the rat and human central nervous system in Table 1.4, overall the distribution of NPY exhibits a characteristic pattern of high levels in forebrain regions such as the hypothalamus, cerebral cortex and the caudate nucleus tending towards lower levels in the direction of the brainstem and spinal cord. High concentrations of NPY-like immunoreactivity (IR) has also been detected in the sympathetic nerve fibres innervating cerebral arteries (Edvinsson et al, 1987). In general, fibres are dense around arteries and few or moderate in number around veins (Uddman et al, 1985).

Central Neuropeptide Y Pathways

Several studies have examined the central distribution (origin and projections) of the NPY-like immunoreactive neurones. Some of the more pertinent systems in the rat brain will be considered. In the hypothalamus, numerous NPY perikarya were found in the arcuate nucleus with a lower number in the paraventricular and preoptic areas (A_{12} and A_{14}). Moderate to

Table 1.4

Distribution of Neuropeptide Y-like Immunoreactivity within Regions of the Rat and Human Central Nervous Systems

Region	Concentration (pmol g^{-1} wet tissue)	
	rat	human
Hypothalamus	730 ± 164	182 ± 21
Cerebral cortex	179 ± 24	120 ± 13
Caudate nucleus	313 ± 69	534 ± 170
Cerebellum	< 0.75	1.3 ± 0.2
Pons	113 ± 23	7 ± 1
Medulla oblongata	15 ± 3	nd
Spinal cord - thoracic	21 ± 3	34 ± 5
Spinal cord - sacral	92 ± 9	47 ± 11

Data taken from Allen et al (1983a; 1984); Adrian et al (1983); Chronwall et al (1985); Gibson et al (1984); nd = not determined.

high densities of NPY fibre networks and terminals were also located in these hypothalamic regions (Chronwall et al, 1985). NPY-positive neurones have been observed to project from the arcuate nucleus to the paraventricular nucleus and from the dorsomedial hypothalamic region to the nucleus tractus solitarius (Bai et al, 1985; Gray and Morley, 1986). The number of NPY perikarya and extent of fibre networks in the cerebral cortex is high especially compared to other peptides previously mentioned. In contrast, the cerebellum contains no NPY-positive cell bodies or terminals in agreement with the low levels of NPY measured by radioimmunoassay techniques (Chronwall et al, 1985).

The medulla and pons embody three major NPY-immunoreactive neuronal systems which closely parallel the catecholamine-containing cell bodies and fibre systems of these regions. These are the A_1/C_1 cell groups of the ventrolateral medulla and the A_2/C_2 cell groups of the dorsal medulla as well as the A_6 region of the locus coeruleus (Chronwall et al, 1985). Indeed, using sequential staining techniques with antisera against NPY and the enzyme markers for noradrenaline (tyrosine hydroxylase) and adrenaline (phenylethanolamine-N-Methyltransferase), immunohistochemical studies have shown colocalisation of NPY with catecholamines in some of these brainstem neurones as well as in other central regions. The extent of the colocalisation depends on the region, pathway and neurotransmitter examined. However, a general feature observed is that only a subpopulation of the neuronal groups contain both NPY and a catecholamine. There is evidence to suggest that NPY is colocalised in the vast majority

of adrenergic cell groups (C_1 - C_3) but is colocalised only in a subset (A_1, A_2, A_4, A_6) of the total noradrenergic cell population. The findings of these immunohistochemical studies in the medulla oblongata (rat, human and rabbit) have been summarised in Table 1.5. These data demonstrate that differential proportions of noradrenergic and adrenergic neurones of the ventrolateral A_1/C_1 and the dorsomedial A_2/C_2 cell groups of the medulla contain NPY-IR. Differences apparently exist between species ostensibly in the A_2 cell group where major colocalisation was only observed in the human medulla. However, methodological differences between studies cannot be excluded. Maps of NPY-IR show that low to moderate density fibre and terminal networks surrounding these cell bodies. In particular, terminals around the A_1/C_1 neurones show a close overlap with catecholamine positive fibres in these regions (Everitt et al, 1984; Harfstrand et al, 1987a).

The involvement of these catecholamine cell groups in central cardiovascular regulation and neuroendocrine function (Section 1.3) also implicates NPY and points to the possibility that NPY may have a modulatory role in this region, interacting with the endogenous catecholamines - a concept that will be examined in subsequent chapters. The postulated involvement of NPY in neuroendocrine and autonomic regulation is further supported by the finding that cells which contain both NPY and catecholamines have been shown to project to the hypothalamus (Sawchenko et al, 1985). Moreover NPY fibre systems and

Table 1.5

Summary of Differential Coexistence Patterns of NPY-like Immunoreactivity in Brainstem Catecholamine Groups

Area	Catecholamine	Coexistence rat	human	rabbit
CVLM	NA (A_1 group)	Yes, major	Yes, minor	Yes, partial
RVLM	A (C_1 group)	Yes, complete	Yes, partial	Yes, major
Dorsal medulla oblongata (NTS; Dorsal vagal complex)	NA (A_2 group)	Yes, minor	Yes, major	No
	A (C_2 group)	Yes, major	Yes, partial	Yes, major
	DA	No	nd	nd
Area postrema	NA	No	nd	nd
Pons, locus coeruleus	NA (A_6 group)	Yes, partial	No	nd

A - adrenaline
NA - noradrenaline
DA - dopamine

major > 75%
partial > 50%
minor > 25%

nd - not determined

CVLM - caudal ventrolateral medulla
RVLM - rostral ventrolateral medulla
NTS - nucleus of the solitary tract

From Data of Everitt et al (1984); Halliday et al (1988); Blessing et al (1986)

terminals, but not cell bodies, have been located in the dorsal horn at all levels of the spinal cord (Gibson et al, 1984; Allen et al, 1984). In particular, NPY-immunoreactive nerve fibres and terminals were evident in the intermediolateral cell columns of the thoracic segment, where they closely parallel, and are possibly associated with, the site of termination of descending catecholamine neurones (Chiba and Murata, 1981; Blessing et al, 1987b; Gibson et al, 1984).

Not all neurones in the regions described contain both a catecholamine and NPY. Indeed the complexity of the situation is highlighted by the finding that a proportion of catecholamine neurones may contain other peptides such as substance P (C_1, C_2), neurotensin, cholecystokinin (C_2 dorsal) or galanin (A_1, A_2, A_6) (Lorenz et al, 1985; Hokfelt, 1984b; 1985; Melander et al, 1986). Moreover, NPY has been shown to be present in neurones containing other putative transmitters, such as somatostatin, FMRF-amide, gamma-amihobutyric acid and serotonin in several discrete locations throughout the central nervous system (Chronwall et al, 1984; Hokfelt et al, 1983a; Hendry et al, 1984; Blessing et al, 1986). Neurons containing NPY alone have been observed in the medulla and hypothalamus (Everitt et al, 1984). In addition to the immunohistochemical evidence of NPY-containing cell bodies and fibre systems, receptor binding studies have shown high affinity binding sites in rat brain membrane preparations, suggesting the existence of NPY receptors (Saria et al, 1984; Unden et al, 1984). These putative receptors have been widely but discretely located in the brain. Areas enriched in both NPY

receptors and fibres have been demonstrated (nucleus of the solitary tract, ventral tegmental area). However, there is evidently a lack of correspondence in the pattern of distribution between the NPY receptors and NPY fibre systems in certain regions. Numerous structures have been observed to have NPY innervation but few or undetectable receptors. This situation exists in the preoptic area, hypothalamus (suprachiasmatic nucleus) and the ventrolateral medulla. In contrast, in areas of dense binding such as the cortical layers I-IV and the area postrema, fibres are either sparsely scattered or not detected (Martel et al, 1986; Nakajima et al, 1986; Harfstrand et al, 1986). The mismatch of receptors and NPY-containing neuronal systems is not restricted to NPY or indeed to peptide transmitters and has been observed to some degree for all categories of transmitters including monoamines and amino acids. This phenomenon, which has been reviewed recently by Herkenham (1987) with several possible explanations propounded, does not detract from the substantial evidence described above suggesting an important function within the central nervous system.

1.5.4 Effects of Neuropeptide Y in the Peripheral Nervous System

Systemic administration of NPY per se has pronounced cardiovascular effects characterised by an increase in arterial pressure and a reduction in heart rate. Other notable peripheral actions of NPY are summarised in Table 1.6.

Table 1.6

Proposed Peripheral Effects of Neuropeptide Y

Peripheral Effects
Increase in systemic blood pressure
Vasoconstriction
Potentiation of vasoconstriction
Presynaptic inhibition of transmitter release
Positive and/or negative chronotropic action
Negative inotropic action
Inhibition of cardiac vagal action
Relaxation of colon smooth muscle and inhibition of colonic motility
Suppression of insulin release
Natriuresis
Reduction of circulating atrial natriuretic factor and vasopressin
Suppression of renin secretion
Enhancement of stimulated thyroid hormone secretion

See text, Gray and Morley (1986) and Higuchi (1989) for details and references

In the peripheral circulation, NPY has been reported to cause vasoconstriction which is unrelated to alpha-adrenoceptors (Lundberg and Tatemoto, 1982). This evidence combined with its observed potent contractile effects in the renal and coronary circulation support the view that a major part of the systemic pressor response to NPY may be mediated by direct vasoconstriction (Allen et al, 1985; Franco-Cereceda et al, 1985). This contractile activity, which can also be observed with the structurally related PYY is typically slow on onset and has a prolonged duration of action. In the intact animal these effects may be related to the relatively long (20 minute) plasma half-life of NPY (Pernow et al, 1987). However NPY has weak contractile effects in a number of isolated peripheral vessels, an effect that is dependent on the vessel (precise segment) and species examined. The rabbit gastro-epiploic artery responds to NPY with a weak contraction whereas the rat femoral artery responds with a stronger contraction (Edvinsson et al, 1984a; Lundberg et al, 1985). NPY can also modify vascular resistance by enhancing the contriction elicited by other agents such as noradrenaline or histamine. Evidence of the functional interaction with the peripheral sympathetic nervous system is supported by a number of studies. In tissues which include the rabbit femoral artery and vein, NPY can act postsynaptically to potentiate the vasoconstriction evoked by nerve stimulation or by noradrenaline (Edvinsson et al, 1984a; Dahlof et al, 1985; Wahlestedt et al, 1985). This potentiating effect is manifest at nanomolar concentrations, far below the concentrations required

to evoke a direct vasoconstrictor response.

NPY can also act presynaptically to reduce the release of noradrenaline from sympathetic nerve terminals (rat vas deferens) indicating the existence of a presynaptic interaction between NPY in the sympathetic nervous system (Stjarne et al, 1986). Consistent with the investigations on isolated tissues, it has been demonstrated that in intact rats the pressor response to alpha-adrenoceptor agonists are potentiated by NPY to a greater extent than the response to sympathetic nerve stimulation (Zukowska-Grojec et al, 1986). This data implies that NPY can enhance pressor responses postjunctionally while attenuating transmitter release by a prejunctional action.

The possible consequences of these diverse actions of NPY at the sympathetic neuroeffector junction may be to improve the transmitter economy, reflected in a reduced noradrenaline demand and an attenuation or shortening of noradrenaline release (Glover, 1985; Stjarne, 1986). It should be considered that NPY may not produce all three effects at all neuroeffector junctions and it is possible that the distribution of receptor subtypes may be the limiting factor. Indeed, in contrast to the post junctional effects of NPY previously discussed, the C-terminal fragment of NPY_{13-36} has been observed to mimic the effects of NPY at the presynaptic receptors in the vas deferens. Thus peripheral post- and prejunctional receptors are suggested by some to be structurally distinct and have been classified as Y_1 and Y_2 respectively, NPY_{13-36} being selective for Y_2 receptors

(Wahlestedt et al, 1986). These subtypes of NPY receptors, based of fragment selectivity, are not however observed in all systems and their existence may only be established with certainty following the development of selective antagonists (Lundberg, 1988).

1.5.5 Effects of Neuropeptide Y in the Central Nervous System

In accordance with its contractile activity in peripheral blood vessels, NPY evokes a potent vasoconstriction in cerebral blood vessels of several species which is not modified by adrenoceptor blockade. Microapplication of NPY around cerebral arteries and arterioles in the anaesthetised cat produced a constriction of commensurate magnitude as that produced by noradrenaline. However the response to NPY was more prolonged. Perivascular application of NPY also constricts cerebral veins but generally higher concentrations are necessary to produce a significant effect (Edvinsson et al, 1983; 1984b). Although NPY potentiates the vasoconstriction evoked by transmural nerve stimulation and exogenous noradrenaline in numerous peripheral arteries, this effect was not observed in cerebral vessels of the rat. Furthermore NPY did not significantly alter the nerve stimulated release of noradrenaline from cat cerebral vessels. Thus it would appear that a direct vasoconstriction is the predominant response to NPY in cerebral blood vessels (Edvinsson et al, 1987). In addition to its effect on cerebral blood vessels, localised injections of NPY into brain parenchyma or administration into the cerebral ventricles elicits changes in a

variety of behavioural and physiological parameters (Table 1.7). A role for NPY in the regulation of food and water ingestion has been implicated by several studies. Administration of NPY into the paraventricular nucleus results in an increased food and water ingestion as well as reducing the time the animal spends on grooming (Stanley and Leibowitz, 1984). The former behavioural response was associated with a preference for carbohydrates and was localised to the hypothalamus (Stanley et al, 1985). Although NPY is colocalised with catecholamines in a number of discrete neuronal systems which include brainstem catecholaminergic projections to the paraventricular nucleus of the hypolthalamus, NPY produces its effects on ingestive behaviours independently from noradrenaline (Gray and Morley, 1986). NPY also had marked effects on the hypothalamo-pituitary axis, including modulating the release of gonadotropin-releasing hormone and subsequently luteinizing hormone secretion with suppressed lordosis in the estradiol and progesterone primed ovariectomised rat (Gray and Morley, 1986). Other noteworthy effects of centrally administered NPY include behavioural and EEG signs (Synchronisation) of sedation similar to the effects produced by the alpha$_2$-agonist clonidine (Fuxe et al, 1983).

The diverse nature of the effects produced by NPY is most likely a reflection of its widespread distribution in the central nervous system. Potentially the most interesting and important findings however, are that NPY is localised in central regions associated with cardiovascular regulation (Section 1.5.3). Moreover it has been shown that administration of this peptide

Table 1.7

Reported Central Effects of Neuropeptide Y

Central Effects
Increased food intake
Increased drinking
Enhancement of gonadotropin-releasing hormone
Modulation of luteinizing hormone
Reduction of circulating growth hormone
Reduction of circulating thyrotropin
Elevation of circulating prolactin
Elevation of circulating vasopressin
Shift of circadian rhythms
Hypothermia
Bradypnea
Sedation
Synchronization of EEG activity
Decreased lordosis
Decreased grooming
Suppression of sexual behaviour
Inhibition of memory
Reduction in blood pressure and heart rate

See text, Gray and Morley (1986) and Higuchi (1989) for details and references

into the cerebrospinal fluid results in alterations in a variety of cardiovascular parameters including reduction in blood pressure and heart rate (Fuxe et al, 1983; Macrae and Reid, 1988). The putative role of NPY in central cardiovascular function is the subject of examination in subsequent chapters within this thesis and will be discussed in detail therein.

1.5.6 Proposed Mechanisms of Action for Neuropeptide Y

For the purposes of this thesis, the present account will confine itself to the mechanisms underlying the purported cardiovascular functions of NPY. Several studies have shown that the vasoconstrictor response produced by NPY can be blocked by calcium channel antagonists both in vitro (peripheral and central blood vessels) and in vivo suggesting that an influx of extracellular calcium is necessary for the response (Lundberg and Tatemoto, 1982; Edvinsson et al, 1985). In contrast to this effect on vascular smooth muscle, calcium entry blockers do not influence the direct contraction evoked by NPY in the mouse vas deferens implying that the mechanism may be tissue dependent (Stjarne et al, 1986). The potentiating effect of NPY in peripheral blood vessels has been reported by Nield (1987) to reflect a general change in smooth muscle reactivity (partial depolarisation). While some believe this effect is caused by NPY increasing the permeability to Ca^{2+} of partially depolarised smooth muscle, others purport that the potentiation depends on Na^{+} but not Ca^{2+} influx and that an intracellular sequestered pool of Ca^{2+} participates (Glover, 1986; Wahlestedt et al, 1985).

Stjarne et al (1986) proposed that the presynaptic action of NPY on transmitter release at the sympathetic neuroeffector junction is associated with a depression of the stimulus-secretion coupling (invasion of varicosities by action potentials) rather than an effect on the secretory process itself. In central nervous tissue the inhibition of neurotransmitter release by NPY may be a consequence of the inhibition of neuronal Ca^{2+} currents (Walker et al, 1988a; Colmers et al, 1987).

It is of considerable interest that NPY has been shown to attenuate the forskolin-induced formation of cAMP or adenylate cyclase in several tissues which include some peripheral and cerebral blood vessels (Lundberg et al, 1988; Fredholm et al, 1985; Haggblad and Fredholm, 1987). This inhibitory action of NPY on cAMP formation may mediate at least some of the reported functional activities of the peptide. Evidence favouring the direct involvement of the other second messenger system, phosphoinositide hydrolysis, in this peptide's action on smooth muscle is less convincing. Although inositol phosphates may participate in the enhancement of NPY on agonist-induced contractions (Haggblad and Fredholm, 1987; Lundberg et al, 1988).

1.6 Endothelin-1

1.6.1 Identification of Endothelin-1

Endothelin-1 (ET-1) is an endothelial cell-derived peptide, which was isolated and sequenced by Yanagisawa and colleagues (1988a) from the culture medium of porcine aortic endothelial

cells. Despite the very recent discovery of this peptide, substantial progress has been made concerning its identity and origins. Indeed the endothelin gene was cloned and sequenced prior to the pharmacological characterisation of the peptide. The peptide consists of 21 amino acid residues with free amino and carboxytermini plus two sets of intrachain disulfide bridges (Figure 1.6). Sequence analysis of cloned endothelin-1 cDNA demonstrated that a precursor peptide of 203 amino acids is synthesised which contains a signal sequence which is proteolytically cleaved to produce the 39 (porcine) or 38 (human) amino acid intermediate big endothelin-1. This precursor is then apparently processed to mature endothelin (ET-1) by a putative 'endothelin converting enzyme'. The sequences of mature ET-1 in porcine and human are identically conserved (Yanagisawa et al, 1988a). The presence of mRNA encoding the prepro-form of endothelin in vascular endothelium and the lack of secretory granules in which the peptide could be stored in this tissue suggest that endothelin is produced by de novo synthesis with processing analagous to many hormones and neuropeptides. Moreover the level of mRNA expression can be influenced by vasoactive agents such as thrombin and adrenaline (Yanagisawa et al, 1988a).

The existence of a 'family of endothelins' has been indicated by the finding that three genes related to ET-1 are present in the human porcine and rat genomes. The amino acid sequences of the 21 amino acid peptides predicted by the three

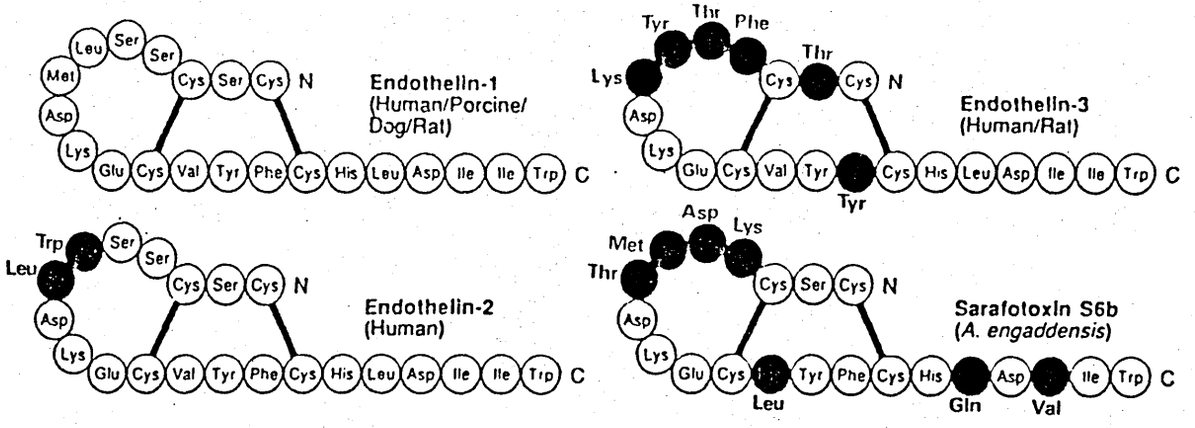


Figure 1.6

Amino acid sequences of peptides from the endothelin family.

Filled circles represent amino acid residues which differ from those in ET-1. Since ET-2 and ET-3 have not yet been identified at the peptide level, the bond topologies and the chain lengths of these predicted peptides have been drawn by comparison with ET-1.

Adapted from Yanagisawa and Masaki (1989).

genes in humans differed at several amino acid residues. These peptides have since been designated endothelin-1 (ET-1 or the original porcine/human ET), endothelin-2 (ET-2, with two amino acid substitutions from ET-1 and endothelin-3 (ET-3, with six amino acid substitutions). ET-3 is identical to a sequence that was described earlier in the rat genome (Figure 1.6, Yanagisawa and Masaki, 1989a). The endothelin group display a striking sequence homology with a family of 21-residue peptide toxins (safarotoxins) newly purified and sequenced from snake venom and has prompted some investigators to suggest a common evolutionary origin (Figure 1.6). A relationship between these mammalian and snake peptides is also indicated by their similar functional activities and the finding that they bind competitively to the same specific receptor in certain tissues (Kloog and Sokolovsky, 1989). It is possible that the three endothelin isopeptides are produced in many mammalian species and may have different functions, possibly by acting at different receptors.

1.6.2 Peripheral Distribution of ET-1

Following the initial report by Yanagisawa et al (1988a) showing that genetic markers (mRNA) for the ET-1 precursor were present not only in cultured endothelial cells but also in the porcine aortic intima in situ, a number of studies have investigated the location and distribution of this novel peptide. Unlike other peptides including neuropeptide Y which have been localised mainly by radioimmunoassay and immunohistochemical techniques, studies concerning the distribution of the endothelin

peptides to date have generally involved indirect methods of locating the radiolabelled binding sites using autoradiographic techniques or detecting the genetic markers for the peptide. Although ET-1 has been detected at the peptide and at the mRNA level in vascular endothelial cells, the other cognate peptides ET-2 and ET-3 have not been observed in this tissue. Indeed the expresion of ET-2 has not yet been conclusively demonstrated in any tissues (Yanagisawa et al, 1989a). Several studies have demonstrated a wide distribution for ET-1 binding sites. In cultured rat vascular smooth muscle cells, a single class of high affinity binding sites for ET-1 has been described, supporting a role for this peptide in vascular reactivity. Autoradiographic studies with iodinated ET-1 have also shown specific binding sites of ET-1 in the media of a variety of blood vessels. The binding sites in these blood vessels appeared to be more dense towards the adventitia than on the portion of the smooth muscle closest to the lumen (Hirata et al, 1988; Koseki et al, 1989a).

Systemic administration of iodinated ET-1 to rats labelled a variety of tissues with high concentrations found in the lungs, kidneys, adrenal medulla and liver, and lower densities observed in heart muscle, stomach, spleen, large blood vessels and renal arteries. The autoradiographic features of these tissues labelled in vivo were qualitatively similar to the individual labelling of tissues in vitro, however the relative densities of ET-1 labelling differed (Koseki et al, 1989a). Binding sites for ET-1 in peripheral tissues have also been determined in the human, pig, mouse and monkey (Hoyer et al, 1989; Davenport et al,

1989). The distribution of binding sites in some tissues was not homogeneous and was not exclusively associated with vascular smooth muscle. In the kidney for instance, the medulla displayed higher densities of sites than in the cortex. This suggests that ET-1, as well as influencing blood vessel functioning, may exert other physiological effects in these tissues which are not directly related to vascular reactivity (Koseki et al, 1989a; Davenport et al, 1989).

1.6.3 Central Distribution of ET-1

Studies to determine the distribution of ET-1 in the brain after systemic administration of the radiolabelled peptide in the rat have shown that the peptide bound only to the circumventricular structures of the median eminence of the hypothalamus and subfornical organ located outside the blood brain barrier and the choroid plexus (Koseki et al, 1989a). Although the peptide does not appear to cross the blood brain barrier, incubation of brain sections with the radiolabelled peptide revealed that as well as brain blood vessels containing ET-1 binding sites, many discrete parenchymal regions throughout the rat and human brain were labelled with the peptide. High levels of binding sites were found in the cerebellum and hypothalamus with lower levels in the neocortex (Table 1.8). Specific binding sites were also found in nuclei-associated with cardiovascular regulation in the medulla, pons and the spinal cord of the rat (Koseki et al, 1989a; 1989b; Hoyer et al, 1989; Jones et al, 1989; Davenport et al, 1989). The pattern of

Table 1.8

Autoradiographic Distribution of [^{125}I]-ET-1 Binding Sites in Selected Regions of the Rat and Human Brain

Brain Area	Rat	Human
Cerebellum	882	776
Hypothalamus	727	624
Substantia nigra	651	nd
Thalamus	517	nd
Caudate putamen	255	nd
Neocortex	193	nd
Brain vessels	nd	163

Data taken from Hoyer et al, 1989 and expressed as fmol mg^{-1} tissue.

nd = not determined

distribution of ET-1 binding sites in the central brain areas is distinct from other known peptides including vasopressin and neurotensin (Yamamura et al, 1983; Quirion et al, 1982). Moreover the non-homogenous pattern of distribution of ET-1 indicates that in addition to possible vascular activity ET-1 may act in the central nervous system in a neurotransmitter or neuromodulatory capacity to regulate a variety of neuronal functions. ET-3-like immunoreactive material has been located in porcine brain homogenates and ET-1-like immunoreactivity demonstrated in motor neurones and primary sensory neurones, suggesting a neural function for these isopeptides (Yanagisawa and Masaki, 1989a). A complete definition of the presence and location of ET-1 (and cognate peptides) in the central nervous systems, requires further work.

1.6.4 Effects of ET-1 in the Peripheral Nervous System

ET-1 evokes a potent and sustained contraction in isolated arterial and venous smooth muscle preparations in almost all animal species and vascular regions examined. The effective dose of ET-1 on these blood vessels varies, with renal and pulmonary arteries being the most sensitive (Yanagisawa et al, 1988a; Shigeno et al, 1989; Tomobe et al, 1988). In porcine coronary artery strips the dose-response relation of the contractile activity of ET showed that the maximum tension developed was comparable to those of potassium chloride-induced contractions. The dose at which ET-1 was 50% effective (EC_{50}) in this tissue was 4.5×10^{-10}M, at least one order of magnitude lower than the

reported values for neuropeptide Y, angiotensin II and vasopressin (Edvinsson, 1986; Regoli et al, 1974; Altura, 1970). In addition EC_{50} values in many other arteries and veins were also within this range, suggesting that ET-1 is one of the most potent vasoconstrictors known to date with important implications in vascular regulation (Yanagisawa et al, 1988a).

Intravenous bolus injection of ET-1 in rat provokes a transient dose-related fall in blood pressure followed by a sustained dose-dependent elevation in arterial pressure. This biphasic response has been associated with differential changes in peripheral vascular resistance (Yanagisawa et al, 1988a; 1988b; Han et al, 1989). A characteristic feature of the response to systemically administered ET-1 is the long duration of the pressor phase (2-3 hours) at high doses. The other members of the endothelian family (ET-2, ET-3) also have constrictor properties. ET-3, in general, has much weaker contractile effects than ET-1 or ET-2 both in isolated tissues and in vivo. Furthermore ET-3 has been observed to produce a greater initial transient depressor response in vivo compared to the other isopeptides (Inoue et al, 1989). The depressor response to ET-1 is also observed in isolated perfused pre-contracted rat mesenteric arteries and can be attenuated by oxyhaemobglobin indicating that the response may be mediated by the release of endothelium-derived relaxing factor (de Nucci et al, 1988). Prostacyclin, another potential vasodilator and thromboxane A_2 were also reported by de Nucci et al (1988) to be liberated from the perfused isolated rat lung. However the

importance of endothelium-derived relaxing factor and the other agents mentioned in the response to ET-1, in vivo remains to be established.

Although ET-1 was originally described as a vasoconstrictor agent, this peptide has since been recognised to possess a wider variety of activities. ET-1 can induce the proliferation of vascular smooth muscle cells, which may have important implications in the pathogenesis of atherosclerosis (Komura et al, 1988). Direct effects of ET-1 on cardiac function have been reported. In particular, positive inotropic and chronotropic actions, on myocardium of the isolated guinea pig atria have been observed (Ishikawa et al, 1988a; 1988b). Another cardiac effect which may have physiological significance is the secretogogue action of ET-1 on atrial natriuretic peptide in isolated *atria* (Hu et al, 1988). ET-1 is also reputedly involved in the regulation of renal function. This is based on the finding that ET-1 administered into the renal artery of dogs in vivo evoked a pronounced decrease in renal blood flow accompanied by a reduction in glomerular filtration rate. These actions could potentially lead to the stimulation of renin secretion and thus to an increase in blood pressure. However, paradoxically ET-1 has also been observed to inhibit renin release from isolated glomerular preparations (Yanagisawa and Masaki, 1989b).

In a more recent and very interesting report by Tabuchi et al (1989) low doses of ET-1 inhibited the release of nerve-

stimulated noradrenaline from presynaptic nerve endings in the rat mesenteric artery. At higher doses in the same tissue this peptide was also observed to enhance the pressor response to nerve stimulation. These differential effects of ET-1 are similar to the results discussed previously for NPY at the sympathetic neuroeffector junction. Thus, as for NPY, in addition to a direct vasoconstrictor response, the above results support a role for ET-1 in the regulation of local vascular tone in association with the sympathetic nervous system.

1.6.5 Effects of ET-1 in the Central Nervous System

The pervasive distribution of specific high affinity binding sites for ET-1 in the brain advocates an involvement of this peptide in a wide variety of central functions. Since the binding sites for this peptide were only identified very recently, there are few published studies on the potential central activities of this peptide. However, a putative role in the regulation of cerebral vascular function is inferred by the presence of ET-1 binding sites on brain blood vessles (Hoyer et al, 1989). In addition, the non-homogenous pattern of distribution in parenchymal tissue supports a neuronal function for this peptide either as a neurotransmitter or a neuromodulator. Indeed, very recent evidence which may support both a central neuronal and vascular function for ET-1 will be considered in the context of the studies in Chapter seven which investigates the potential central cardiovascular actions of ET-1.

1.6.6 Possible Mechanism of Action

The original hypothesis for the contractile activity of ET-1 in isolated tissues at least, was the influx of Ca^{2+} through voltage-dependent calcium channels in smooth muscle. This view was based on the finding that vasoconstriction produced by ET-1 in the porcine coronary artery was inhibited dihydropyridine calcium entry blocker or in the absence of extracellular calcium (Yanagisawa et al, 1988a). Furthermore, ET-1 markedly augments dihydropyridine-sensitive voltage-dependent Ca^{2+} channel currents in whole-cell clamped smooth muscle cells from porcine coronary artery suggesting a close association with the activation of the Ca^{2+} channel (Goto et al, 1989). However, in the cultured vascular smooth muscle cell of the rat, the binding of radiolabelled ET-1 was not displaced by dihydropyridine calcium entry blockers, implying that the specific receptors for ET-1 are distinct from those of the calcium channel antagonists (Hirata et al, 1988). In the same report, these authors further demonstrated that ET-1 produced an initial transient as well as a sustained increase in intracellular Ca^{2+} concentration in vascular smooth muscle cells, with only the second phase of the Ca^{2+} elevation sensitive to calcium channel antagonists. The initial ET-1 stimulated increase in intracellular Ca^{2+} levels may be mediated by an increase in inositol phosphate production which was also observed in vascular smooth muscle cells following ET-1 (Marsden et al, 1989). It has been postulated that this initial elevation in inositol phosphate production mediates an influx of

Ca^{2+} ions resulting in the secondary sustained elevation of Ca^{2+} observed in the vascular smooth muscle cell (Van Renterghem et al, 1988). Therefore it appears that in some tissues, although ET-1 does not act directly on the dihydropyridine-sensitive Ca^{2+} channel, a close association with these voltage operated channels exists.

In other systems ET-1 has been observed to act almost completely independently of the voltage-dependent Ca^{2+} channel. In the coronary artery strip, high concentrations of ET-1 can elicit a contraction which does not require extracellular calcium but is associated with an increase in inositol trisphosphate as well as another product of phospholipid breakdown, 1, 2-diacylglycerol (Kasuya et al, 1989). This data suggests that ET-1 can evoke a potent vasoconstriction which may involve intracellular calcium mobilisation (inositol trisphosphate) and stimulation of protein kinase C by 1,2-diacylglycerol. These second messengers could potentially lead to the activation of the contractile machinery. This view is consistent with the previous work of Auguet et al (1988), who observed that in the rat aorta, the contraction elicited by ET-1 did not involve the voltage-dependent calcium channels. Thus, the increased intracellular Ca^{2+} levels and vasoconstriction subsequent to ET-1 does not necessarily involve direct activation of voltage-dependent calcium channels and in some systems ET-1 can act independently of extracellular calcium.

CHAPTER TWO

GENERAL METHODS AND MATERIALS

Chapter Two

General Methods and Materials

2.1 Introduction

This chapter details the general methods ulitised in this thesis, more specific methodology is described within the individual chapters. All animal studies were carried out using male, normotensive, Wistar-Kyoto rats (250 - 350 g).

2.2 Blood Pressure and Heart Rate Measurement in Chronically Anaesthetised Rats

Animals were anaesthetised with pentobarbitone sodium (60 mgs kg^{-1}, i.p.) and a polyethylene catheter, filled with heparinised saline (8 units ml^{-1}), placed in the abdominal aorta or femoral artery. The catheter was then sealed and tunneled s.c. to be exteriorised at the back of the neck between the shoulder blades. Patency was ensured by filling the catheter with a polyvinyl pyrrolidone saline solution (0.5 mgs ml^{-1}) containing 200 units ml^{-1} of heparin. The animals were then allowed to recover from the surgery for a period of 24 hours. On the study day anaesthesia was induced with a combination of urethane (1 g kg^{-1}, i.p.) and alpha chloralose suspension (60 mgs kg^{-1}, i.p.) and maintained with urethane (300 mgs kg^{-1}, i.v.). Rectal temperature was kept constant at 37°C with an automated heating blanket. After a stabilisation period of one hour, arterial pressure (mmHg) was recorded via the catheter by a Statham pressure transducer connected to a Grass Polygraph (7D) and heart rate was monitored by a cardiotachograph triggered by

the arterial pulses.

2.3 Intraparenchymal Injections into the Medulla Oblongata of Anaesthetised Rats - Coordinates

Anaesthesia was maintained and arterial blood pressure and heart rate and temperature monitored as previously described in section 2.2. The rats were placed in a David Kopf stereotaxic frame for small animals. A midline incision was made from the level of bregma to the lambdoid suture, the fascia retracted and the skull exposed.

For microinjections into the caudal ventrolateral medulla, the incisor bar was set at 5 mm above the intra-aural line (IAL) using the following stereotaxic coordinates: 4.5 mm rostral to the IAL; 1.7 mm lateral to the midline and 9.9 mm ventral to the cerebellar surface. The rostral ventrolateral medulla was located with the incisor bar set at 5 mm below the IAL and using the following coordinates: 3.5 mm rostral to the IAL; 2.5 mm lateral to the midline and 8.5 mm ventral to the cerebellar surface (Paxinos and Watson, 1986). A small craniotomy (2 mm diameter) was performed using a standard dental drill with size 16 burr. An incision was then made in the dura using the tip of an 18 g needle. Unilateral microinjections of drug or vehicle (0.9% saline), were delivered via a 30 g needle using a 1 µl microsyringe, mounted on a micromanipulator. All injections were delivered in a total volume of 0.1 µl over a 20 second interval.

Pilot experiments were performed for both sets of coordinates to check the accuracy of the needle placement. This

involved stereotaxic microinjection of 0.1 μl methylene blue followed by histological examination of brain sections (see following section). It was demonstrated that the stain never diffused beyond a radius of 0.05 mm from the point of injection in the coronal plane and 0.2 mm in the saggital plane.

2.4 Histology

At the end of each experiment the animals were killed by an overdose of pentobarbitone sodium. The brains were then fixed by transcardiac perfusion with FAGLU (formaldehyde 4% and gluteraldehyde 0.5% in 0.1 M sodium phosphate buffer pH 7.0), excised and 50 μm sections cut and mounted for verification of the injection site. Needle tracts were clearly visible even in unstained sections.

Figures 2.1 and 2.2 illustrate the site of injection for the caudal and rostral ventrolateral medulla, respectively. Experiments where the injection site was not within the caudal or rostral areas were excluded from the study.

2.5 Blood Pressure and Heart Rate Measurement in Conscious Rats

The animals were anaesthetised with the short-acting anaesthetic methohexitone sodium (60 mg kg^{-1}, i.p.). Catheters were then placed in the femoral artery (and vein if necessary), the wound infiltrated with the local anaesthetic lignocaine and the catheters exteriorised as described in section 2.2. A small jacket was fitted to the animal and the catheters passed through

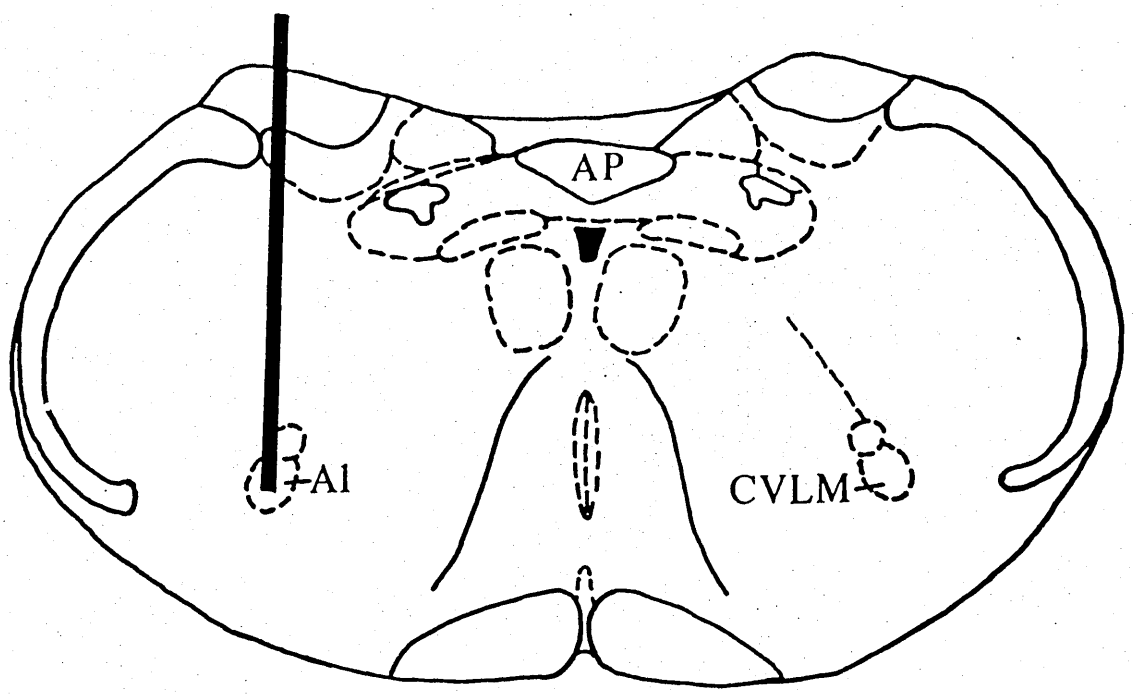


Figure 2.1

Diagram illustrating the site of microinjection in the caudal ventrolateral medulla (CVLM) of the rat.

A_1 = A_1 adrenaline cell group as described by Hokfelt *et al*, (1974).

AP = area postrema

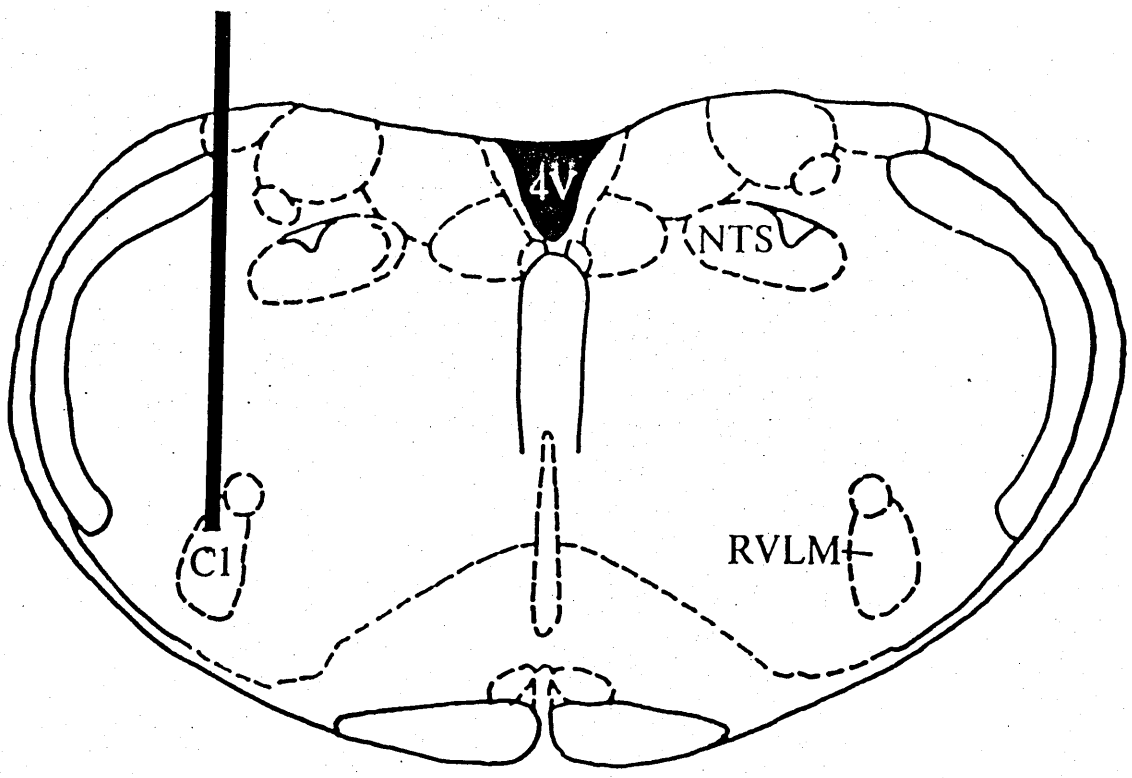


Figure 2.2

Diagram illustrating the site of microinjection in the rostral vento-lateral medulla (RVLM) of the rat.

C_1 = C_1 noradrenaline cell group as described by Dahlstrom et al (1964).

NTS = nucleus of the solitary tract

4V = fourth ventrical

an attached metal spring plus swivel to protect the catheters and allow the animal unrestrained movement. A recovery period of three hours was allowed before continuation of the study. Arterial pressure and heart rate were monitored as discussed previously in section 2.2.

2.6 Intracisternal Cannulation

Animals were anaesthetised with the short acting anaesthetic methohexitone sodium (60 mgs kg^{-1}, i.p.). The animal's head was fixed in a David Kopf stereotaxic frame with the incisor bar set at 3.3 mm below the intra-aural line so that the dorsal surface of the skull was horizontal. A midline incision was made from bregma to the lambdoid suture, the fascia retracted and the skull exposed. Using an electric hand drill and a dental burr, two holes were drilled; one drilled partially through the skull for placement of a steel self-tapping screw for anchorage of a dental cement and one drilled entirely through the skull for placement of the cannula (Figure 2.3). The latter was positioned on the midline 1 mm rostal to the lambdoid suture. The guide cannula was then advanced caudally 7 mm, at an angle of about 45^{o} to the horizontal, so that the bevelled surface of the cannula tip was lying approximately horizontal above the surface of the medulla. Acrylic dental cement was applied around the screw and cannula to fix the cannula to the skull. The cement was allowed to harden, the cannula tested to ensure that the cerebral spinal fluid had been accessed by the cannula. This was achieved by attaching a saline filled tube to a needle which was then inserted into the

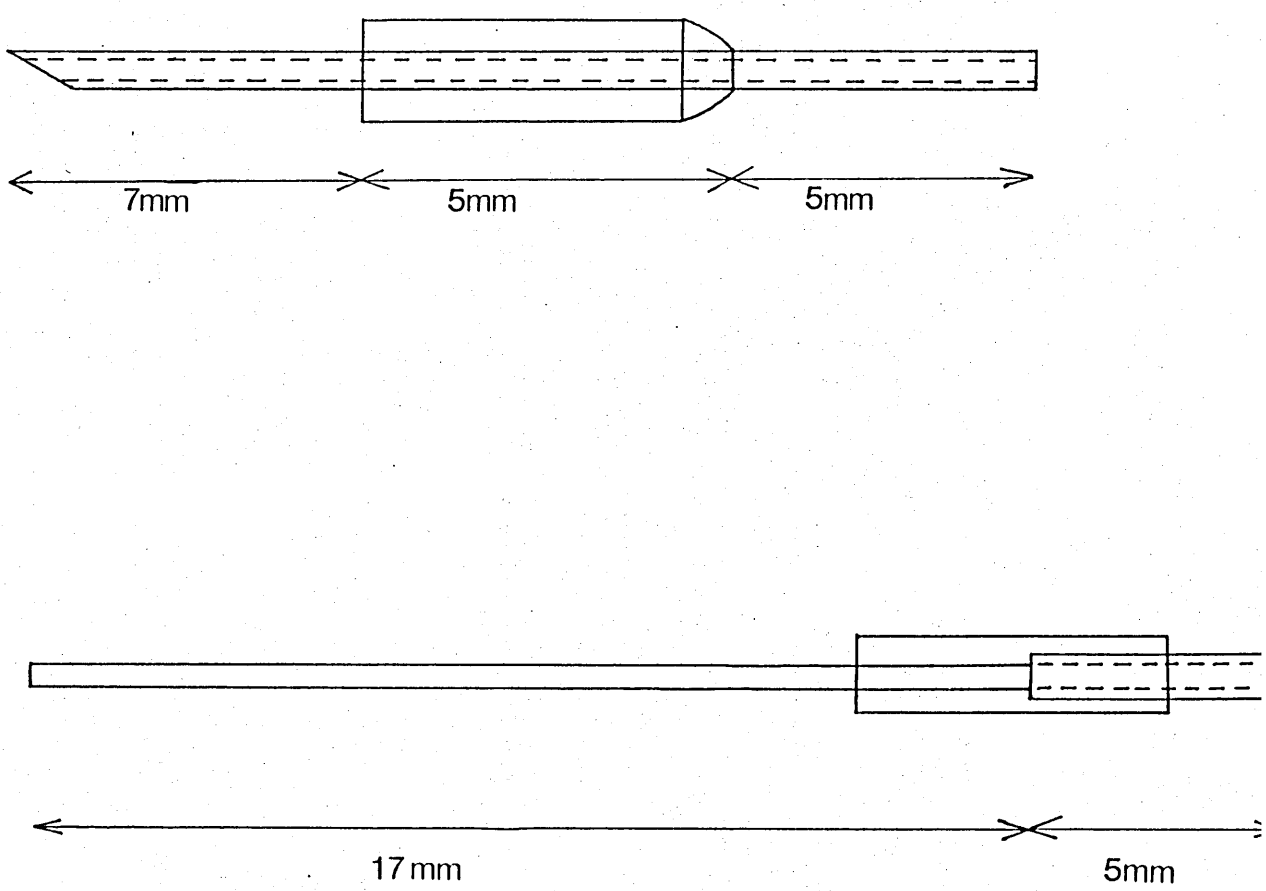


Figure 2.3

Diagram showing dimensions of the intracisternal cannula and stylette.

guide cannula. When the tube was raised and lowered the meniscus should rise and fall. The cannula was then sealed with a stylette (Figure 2.3), cicatrin powder (antibiotic) and the local anaesthetic lignocaine were then applied to the wound before suturing. On completion of surgery ampicillin sodium (30 mg kg^{-1}, i.m.) was given for two days as prophylaxis against infection. The animals were allowed seven days to recover before proceeding with the study.

2.7 Determination of Plasma Catecholamine Levels

Plasma catecholamine levels were determined as an indirect method of measuring sympathetic nerve activity before and after intravenous or intracisternal injection of endothelin in rats.

Arterial blood samples (300 µl) were removed into lithium heparin tubes for the measurement of plasma noradrenaline and adrenaline levels. The blood samples were centrifuged immediately at 1000 g for ten minutes at 4°C, plasma harvested and stored at -70°C. Catecholamine levels were measured by a sensitive radio-enzymatic assay modified from the methods of Peuler and Johnston (1977) with inter-assay coefficients of variation of 10% and 13% for noradrenaline and adrenaline respectively. After separation of plasma, the red blood cells (150 µl) were resuspended in the same volume sterile saline (0.9% w/v) to be returned to the animal after the next sample.

2.8 Measurement of cAMP Accummulation in Brain Slices

2.8.1 Preparation and Preincubation of Brain Slices

Circadian rhythms have been shown to significantly affect basal levels of cyclic adenosine monophosphate (cAMP) in rat brain as well as the synthesis and hydrolysis of this enzyme (Lemmer et al, 1987). Therefore the present experiments were always performed between 9 - 10 a.m. The general protocol for these experiments is shown in Figure 2.4. The animals were killed by decapitation, the brain removed and the medulla or frontal cortex quickly dissected out on ice. The medulla was cut again with a scalpel horizontally into two parallel slabs (3 mm thick). Slices of medulla or cortex (500 x 500 μm) were then cross-cut on a McIlwain tissue chopper. Each brain was transferred to a separate glass tube containing physiological buffer solution A (Table 2.1). Gentle vortexing was used to separate the slices. Consistent with other studies, the samples were then incubated at 37^{o}C for 30 minutes and gassed with 95% O_2/5% CO_2 (Al-Gadi and Hill, 1985). The slices from two brains were then pooled and transferred to 13 mls of buffer B which consisted of the buffer A solution plus theophylline (1.08 mg ml^{-1}), a phosphodiesterase inhibitor to prevent the hydrolysis of cAMP to 5'-AMP. Where necessary bacitracin (0.1 mg ml^{-1}), and/or ascorbic acid (0.2 mg ml^{-1}) was added to this buffer to prevent the breakdown and oxidation of NPY and isoprenaline, respectively. Four aliquots (2 mls slice/buffer

Brain slices incubated in buffer A at

$37^{o}C$ (30 minutes)

↓

Slices from two brains pooled into buffer B

and incubated at $37^{o}C$ (10 minutes)

↓

Slices aliquoted into 4, allowing 1 basal and 3 treatments

and drugs or vehicle control added (15 minutes)

↓

Reaction terminated ($100^{o}C$/4 minutes)

↓

tissue homogenised (10 seconds)

↓

tissue debris removed by

centrigugation (2800 g/15 minutes/$4^{o}C$)

↓

Supernatant removed and stored for subsequent cAMP

determination

Figure 2.4

General protocol for the incubation of rat brain slices

Table 2.1

Composition of Buffer used in the Incubation of Brain Slices

	mM	g/litre
NaCl	118	6.85
KCl	5	0.37
KH_2PO_4	2	0.27
$MgSO_4/7H_2O$	2	0.49
$NaHCO_3$	25	2.10
EDTA disodium salt	0.002	0.05
Glucose	11	2.00
$CaCl_2$	2.5	2.5 mls of a 1M solution

The pH of the buffer was adjusted to 7.4.

solution) from each pooled sample, which allowed for one control and three treatments, were incubated for a further ten minutes.

2.8.2 Drug Treatment of Slices

At the end of the preliminary incubation stage drugs were added in two stages and incubated for a total of fifteen minutes (Figure 2.4). Specific protocols for the drug additions are given in the appropriate chapters (Section 5.2 and Section 6.2).

Following incubation with drugs, the reaction was terminated and tissue cAMP released by boiling the sample at 100°C for four minutes followed by homogenisation (ten seconds). This procedure for extracting cAMP has been demonstrated by Uhlen and Wikberg (1988) to yield similar recovery of cAMP as the acid extraction protocol described by Kelly and Nahorski (1986). Samples (200 ul) were then taken for protein determination using the method of Lowery and co-workers (1951). Tissue debris was removed by centrifugation at 2800 g for 15 minutes at 4°C and samples of the supernatant taken and stored at -20°C overnight for subsequent cAMP determination the next day (Figure 2.4). However, samples could be stored for at least one month without any significant deterioration in cAMP levels.

2.8.3 Measurement of cAMP Accummulation

Levels of cAMP in the samples were determined using a sensitive radioimmunoassay kit technique (Tovey et al, 1974) The assay is based on the competition between unlabelled cAMP and a fixed quantity of the tritium labelled compound, for binding to a protein which has a high specificity and affinity for cAMP.

Separation of the protein bound cAMP from the unbound nucleotide is achieved by adsorption of the free nucleotide on to coated charcoal, followed by centrifugation. An aliquot of the supernatant is then removed for liquid scintillation counting. The amount of labelled protein-cyclic AMP complex formed is inversely related to the amount of unlabelled cAMP present in the assay sample. Measurement of the protein-bound radioactivity therefore enables the amount of unlabelled cAMP in the sample to be calculated.

2.8.4 cAMP Assay Protocol

The assay protocol is shown in Table 2.2, with all procedures carried out, in duplicate, on ice. After the addition of the appropriate agents, tubes were mixed for about five seconds. The assay tubes were then incubated in a cold room at $2^{o}C$ - $4^{o}C$ for two hours. Fifteen minutes before the end of the incubation time charcoal reagent was stirred continuously to ensure a uniform mixture. The charcoal suspension was then added to all tubes in batches of eight, vortex mixed, and allowed to stand for three minutes. The tubes were then centrifuged at 2800 g for five minutes at $4^{o}C$, to sediment the charcoal. Without disturbing the sediment, a 200 μl sample from each tube was removed and placed in scintillation vials. 10 mls of the scintillent 'Hydroluma' was added to each vial for subsequent determination of radioactivity in a β-liquid scintillation counter.

Table 2.2

cAMP Assay Protocol

Tube No.	Buffer (µl)	Standards (µl)	Unknowns (µl)	(^{3}H) cAMP (µl)	Binding Protein (µl)	
1, 2	150	-	-	50	-	Charcoal blank
3, 4	50	-	-	50	100	Zero dose
5, 6	-	50	-	50	100	1 pmol standard
7, 8	-	50	-	50	100	2 pmol standard
9, 10	-	50	-	50	100	4 pmol standard
11, 12	-	50	-	50	100	8 pmol standard
13, 14	-	50	-	50	100	16 pmol standard
15 etc.	-	-	50	50	100	Unknowns

DETERMINATION OF THE CO-EFFICIENT OF VARIATION (CV) IN BASAL cAMP LEVELS WITHIN SAMPLES

Samples measured after 1, 2, 7 and 30 days of storage

cAMP Levels (pmol/mg protein)

SAMPLE	1(1)	2(2)	3(7)	4(30)	Mean ± S.E. Mean	CV(%)
1	2.47	2.69	2.79	2.42	2.59 ± 0.09	6.8
2	2.14	1.95	2.10	2.15	2.09 ± 0.05	4.4

There is no change in cAMP levels with time

2.8.5 cAMP Assay : Precision and Range

Amersham's cyclic AMP assay kit combines the high specificity and affinity for cAMP of a highly purified and stabilised binding protein with a charcoal separation step. This results in little interference by materials likely to be present in crude extracts (Tovey et al, 1974). The kit provides a rapid, simple and specific method for the determination of cAMP. Typical assay data is shown in Table 2.3 and from this the concentration of unlabelled cAMP in the sample is determined from a linear standard curve (Figure 2.5) and then converted to pmols mg^{-1} protein. The assay has a co-efficient of variation of less than 7% over the range of 0.5 - 14 pmol/50 µl sample.

2.8.6 Preliminary Investigations

As will be discussed later in Sections 5.4 and 6.4 the inhibitory reponse to various agents including α_2-adrenergic agonists has been observed by Duman and Enna (1986) to be more easily detected under conditions of elevated cAMP levels achieved by the direct stimulation of the adenylate cyclase enzyme with the diterpene forskolin. In order to reveal the optimal conditions for this stage, concentration-response and time-response relationships for the effect of forskolin on cAMP production in the medulla slice were determined. The preparation and preincubation of the brain slices was essentially the same as detailed in Section 2.5.1 with the following modifications. The brain slices from four rats were pooled into 28 mls of buffer B. Twelve aliquots (2 mls slice/buffer solution) from the pooled

Table 2.3

Typical Assay Data

Tube No.	c.p.m.	Average c.p.m.	Average c.p.m. - blank	Co / Cx	Standard cAMP pmol/tube
1	121	120	-	-	-
2	119				
3	5611	5605	5485	1	0.0
4	5600				
5	3437	3424	3304	1.66	1.0
6	3411				
7	2528	2552	2432	2.26	2.0
8	2576				
9	1659	1679	1559	3.52	4.0
10	1699				
11	1017	1040	920	5.96	8.0
12	1063				
13	627	645	525	10.44	16.0
14	663				

c.p.m. = counts per minute

Co = c.p.m. bound in the absence of unlabelled cAMP

Cx = c.p.m. bound in the presence of standard or unknown unlabelled cAMP

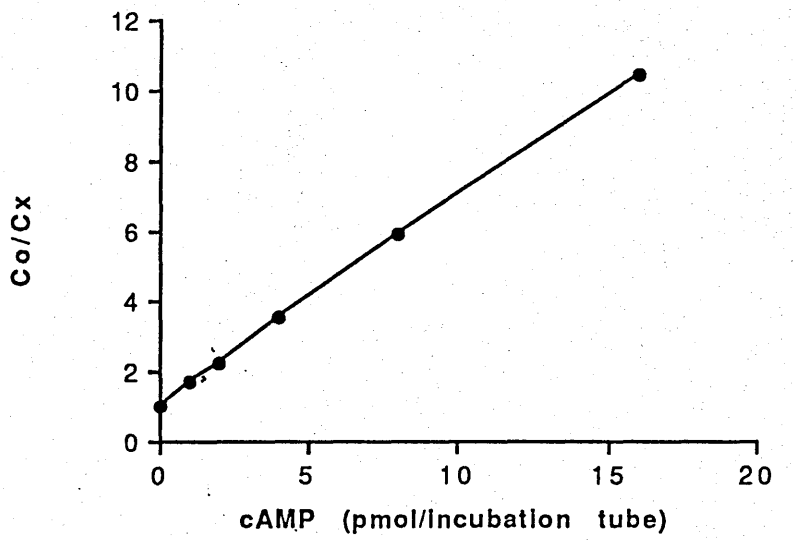


Figure 2.5

A typical cAMP standard curve

CO = radiolabelled cAMP bound in the absence of unlabelled cAMP

CX = radiolabelled cAMP bound in the presence of standard unlabelled cAMP

n = 1 (in duplicate)

sample were taken which allowed six treatment or time samples, each with a separate control. The samples were incubated for a further ten minutes, as described previously (Section 2.5.1). To establish the dose-response relationship to forskolin, the appropriate concentration of forskolin (10^{-8} - 10^{-5}M) was added and incubated for ten minutes. Alternatively, a time-response curve was constructed by incubating the chosen concentration of forskolin (10^{-5}M) for various time intervals (0 - 30 minutes). The reaction was then terminated and cAMP levels measured as described in the previous Sections 2.5.2 - 2.5.4.

Forskolin (10^{-8} - 10^{-5}M) stimulated the cAMP production in the medulla oblongata above control levels in a dose-dependent manner (Figure 2.6). The plateau stage in the dose-response curve was not reached because of solubility problems encountered if higher concentrations of forskolin were used under the present conditions. Ethanol (95%) was used to dissolve forskolin and this solution remains stable for at least one month. Although Robberecht et al (1983) have shown that high concentrations of ethanol inhibit adenylate cyclase activity, the final concentration of ethanol in the present experiments was less than 0.1% which Kitamura et al (1985) have shown does not affect basal adenylate cyclase activity. In accordance with numerous other studies a dose of 10^{-5}M forskolin was used in the following experiments. This represented a concentration which obtained a maximum or near maximum response in studies by Fredholm et al (1986) and Westlind-Danielsson et al (1987; 1988). In medulla slices forskolin (10^{-5}M) evoked an increase in the production of

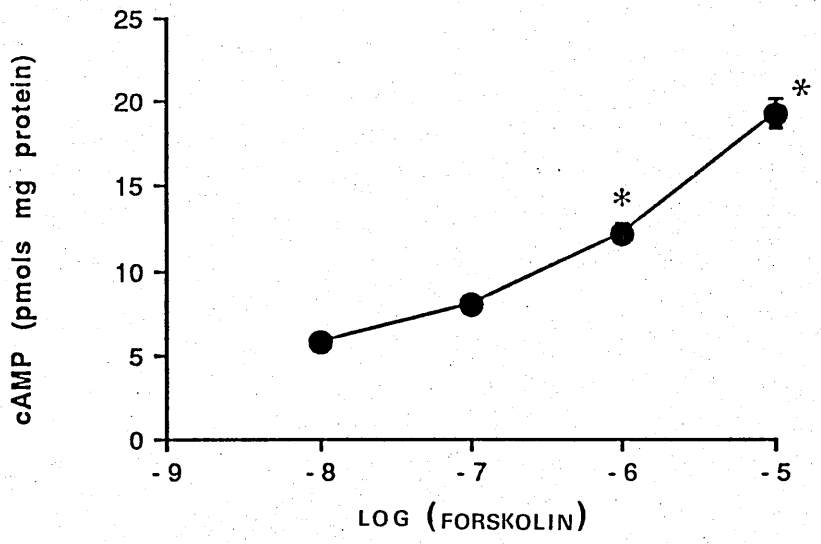


Figure 2.6

Dose-response graph of forskolin (10^{-8} - 10^{-5}M) in slices of the rat medulla oblongata.

Results are expressed as mean ± s.e.mean.

For each point $3 < n < 6$, in duplicate.

* Significant difference from saline control ($p < 0.05$)

cAMP compared with basal values, reaching a stable level after 10 - 15 minutes (Figure 2.7). Consequently, and in agreement with previous investigations (Daly et al, 1982) a ten minute incubation time was chosen for forskolin.

Thus, in the proceeding results (Sections 5.3 and 5.4), where the effects of purported inhibitory substances on cAMP production have been examined, forskolin (10^{-5}M) or vehicle were added and incubated for ten minutes subsequent to an initial five minute incubation with the proposed inhibitory agents.

2.9 Materials

Wistar-Kyoto rats were supplied by Harlan Olac, Bister, England.

Porcine NPY was purchased from Cambridge Research Biochemicals. Clonidine, forskolin, isoprenaline, pertussis toxin and propranolol were obtained from Sigma Chemical Co., Dorset, England. Moxonidine was a generous gift from BDF, Hamburg, Germany. Porcine endothelin-1 was supplied by Scientific Marketing, London. All drugs were dissolved in sterile saline with the exception of forskolin and isoprenaline which were dissolved in ethanol (95% v/v) and ascorbic acid solution (0.1% w/v), respectively. NPY was dissolved in a bovine serum albumin solution (0.5% w/v), aliquoted and stored at -70°C in silicone coated tubes (Sigmacoate). These procedures were carried out to prevent the absorption of the peptide onto the plastic tube. Endothelin-1 was dissolved in sterile saline (0.9% w/v), aliquoted and stored at -20°C in silicone coated

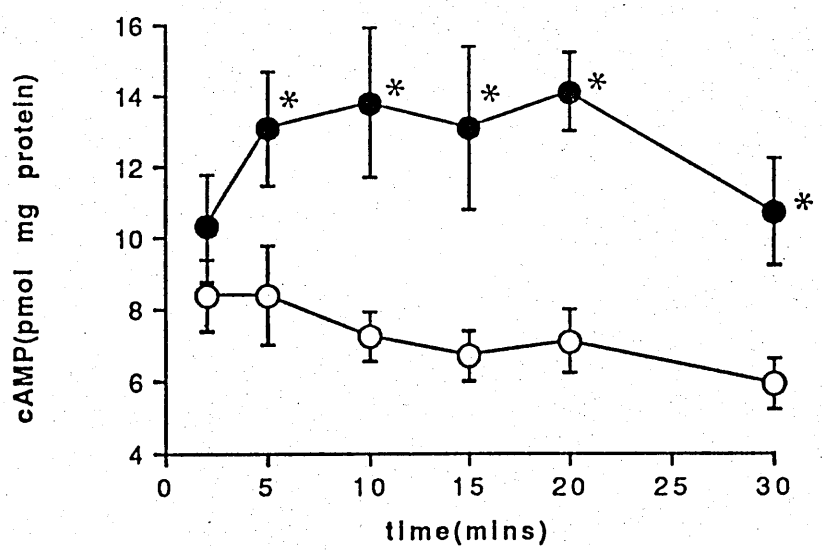


Figure 2.7

Time-response graph of forskolin (10^{-5}M) in slices of the rat medulla oblongata.

Results are expressed as mean ± s.e.mean.

For each point, n = 4 (in duplicate).

○ Basal cAMP levels

● Forskolin-stimulated (10^{-5}M) cAMP levels

* Significant difference from saline control ($p < 0.05$)

tubes.

The contents of the buffers used were obtained from BDH, Dorset, England, with the exception of theophylline and ascorbic acid which were purchased from Sigma and Koch-Light Laboratories, Colnbrook, England.

The anaesthetics used and their U.K. sources are as follows: Methohexitone sodium, Eli Lilly & Co.; Pentobarbitone sodium, May and Baker Ltd.; Urethane, Sigma Chem. Co.; Halothane, I.C.I.; and the alpha-chloralose suspension was a kind gift from Glaxo Group Research, Middlesex. Ampicillin sodium was purchased from Beecham Research, Brentford, England. The heparin solution and polyvinyl pryrrolidone were obtained from Leo Laboratories, Risborough and BDH, England, respectively. The ^{3}H-cAMP radioimmunoassay kit was supplied by Amersham, England. ^{14}C-Iodoantipyrine was also purchased from Amersham. The liquid scintillent Hydroluma was purchased from May and Baker Ltd., England. Equipment used and their sources include a blood gas analyser, Corning, U.K., a Quantimet 970 densitometer, Cambridge Instruments, and Kodak GRL film. The small animal jacket and swivel attachment was obtained from Harvard Apparatus, England.

2.10 Statistics

The majority of the statistical analysis was carried out using Repeated Measures Analysis of Variance and where appropriate, a Bonferroni correction was included for multiple

comparisons. Alternative statistical tests were used in Chapter Seven and are described therein. All results are expressed as mean $\pm$ standard error of the mean (s.e. mean).

CHAPTER THREE

INDIVIDUAL HAEMODYNAMIC ACTIONS OF NEUROPEPTIDE Y AND CLONIDINE AFTER MICROINJECTION INTO THE VENTROLATERAL MEDULLA

Chapter Three

Individual Haemodynamic Actions of Neuropeptide Y and Clonidine after Microinjection into the Ventrolateral Medulla

3.1 Introduction

The central haemodynamic actions of NPY are the subject of continued research, some of which has been stimulated by its co-storage and functional interaction with catecholamines in the peripheral nervous system (Lundberg et al, 1982; Edvinsson et al, 1984a). NPY-like immunoreactivity and binding sites have also been localised in discrete central regions associated with cardiovascular control and thus form a morphological basis for an action of NPY in these regions (Section 1.5.3). Prior to investigating the possible functional interactions between NPY receptors and α_2-adrenoceptors in the medulla oblongata, it was first necessary to establish the individual haemodynamic actions of NPY and the α_2-adrenoceptor agonist, clonidine, in this region.

3.1.1 Central Actions of NPY

Intraventricular administration of NPY has been reported to cause hypotension, bradycardia and bradypnea in the conscious rat (Harfstrand, 1986). In contrast, Vallego and Lightman (1986a) demonstrated an increase in blood pressure after administration of lower doses of NPY into the ventricles of anaesthetised rats. Although the former group attributed the vasodepressor effect of NPY to an action at the medulla oblongata, these results are complicated by the widespread distribution of the peptide

following injection via this route and an action at the level of the hypothalamus cannot be excluded. Indeed NPY has been found to exert varying effects on blood pressure and behaviour depending on the specific site of injection within the hypothalamus. Martin et al (1988) reported an elevation in blood pressure following unilateral injection of NPY in nanomolar quantities, into the posterior hypothalamic nucleus. Microinjection of NPY into the paraventricular nucleus resulted in an increased food intake and a separate stimulation of drinking (Stanley and Leibowitz, 1984). The former behaviour may have cardiovascular consequences since the hypotensive response, subsequent to intraventricular NPY administration, was reported to be counteracted by an NPY-induced feeding action (Harfstrand, 1986). These results however, are confused by the finding that NPY in the paraventricular nucleus NPY can either reduce blood pressure or have no significant effect on blood pressure depending on the report (Harland et al, 1988; Harfstrand et al, 1987a). Furthermore, an increased plasma level of vasopressin was observed in response to NPY microinjected into the hypothalamic supraoptic nucleus of conscious rats (Willoughby and Blessing, 1987). The haemodynamic importance of this elevated plasma vasopressin is in contention, since the pressor response to intracerebroventricular administration of NPY in anaesthetised rats was unaffected by intravenous injection of a specific vasopressin antagonist. However, the finding that this pressor response was attenuated in vasopressin-deficient rats

(Brattleboro), indicates that central vasopressin pathways may be involved in the response to intracerebroventricular injection of NPY (Vallejo and Lightman, 1986a).

The multifarious nature of this peptide is indicated by the above observations and highlights the limitations in the amount of information that can be obtained from injections into the cerebrospinal fluid. Thus, the cardiovascular effects of intracisternal injection of NPY, which initially concentrates the peptide around the brainstem regions are also difficult to interpret. Injection by this route has been shown to reduce blood pressure and heart rate (Macrae and Reid, 1988; Fuxe et al, 1983). However, Petty et al (1984) failed to demonstrate a cardiovascular response to intracisternal injection of NPY.

The anaesthetic state and species of the animals used in these studies may determine the direction of the response to NPY. Alternatively, the variation in the reported haemodynamic effects may also arise from the concentration of NPY used as well as the indiscriminate circulation of the peptide resulting from the volume of injectate or solvent used. These experiments, although necessary starting points, demonstrate that further refinement of the injection technique is required to define the nuclei mediating the central cardiovascular actions of NPY. The specific areas chosen for such investigation were the C_1 group of adrenaline containing neurones in the rostral ventrolateral medulla (RVLM) and in the caudal ventrolateral medulla (CVLM), the noradrenergic A_1 neurones (Hokfelt et al, 1974; Dahlstrom et al, 1964). In addition to being important centres for the

tonic and reflex regulation of blood pressure per se, these areas contain NPY-like immunoreactivity in the cell bodies and terminals, as discussed in previous sections, propounding a functional role for NPY.

3.1.2 Central Actions of Clonidine

Clonidine is a centrally acting hypotensive and bradycardic agent. On the basis of transection experiments and radioligand binding data these haemodynamic effects are considered to be mediated primarily through alpha$_2$-adrenoceptors located in the medulla oblongata (Schmitt and Schmitt, 1969; Unnerstall, 1984), although as shown in Table 3.1, other areas have been implicated. The mechanism of action has been studied extensively in both human and experimental animals with a number of specific nuclei in the medulla proposed as the most likely sites of action. Rockhold and Caldwell (1979) observed that the fall in blood pressure and heart rate, subsequent to intravenous clonidine administration in conscious spontaneously hypertensive rats, was reduced following bilateral electrolytic lesions of the nucleus of the solitary tract (NTS). In contrast, in anaesthetised cats, other investigators have been unable to confirm, by lesioning studies, the purported involvement of the NTS in the central actions of clonidine (Antonaccio and Halley, 1977). However, unilateral microinjection of clonidine (1.25 - 40.0 nmol) into the NTS resulted in hypotension and bradycardia in anaesthetised rats which was attenuated by NTS pretreatment with yohimbine, an α_2-adrenoceptor antagonist (Rockhold and Caldwell, 1980).

Table 3.1

Proposed Sites of the Antihypertensive Action of Clonidine in the Central Nervous System

Region	Reference	Comment
Forebrain : Hypothalamus	Struyker et al (1974)	High doses required to elicit effect, generally pressor response observed (Porter et al, 1985). Hypotensive response to clonidine unaltered by removing forebrain (Schmitt and Schmitt, 1969)
Pons : Locus Cœruleus	Zandberg et al (1979)	Microinjection of clonidine elicits hypotension and bradycardia. In contrast, transection experiments caudal to the pons had no significant effect on hypotensive response to i.v. clonidine (Schmitt and Schmitt, 1969)
Spinal Cord	Cabot and Guyenet (1981)	Clonidine inhibits discharge of preganglionic sympathetic neurones of the intermediolateral cell column. However, in spinal transected rats, with maintained blood pressures, clonidine has no effect (Misu et al, 1982)
Medulla Oblongata	See text	See text

The ventrolateral medulla has also been investigated as a potential site of action of clonidine. Punnen et al (1987) demonstrated that microinjection of idazoxan, an α_2 adrenoceptor antagonist, into a pharmacologically defined pressor area in the ventrolateral medulla, attenuated the reduction in blood pressure and heart rate, evident in anaesthetised rats, following intravenous administration of clonidine. More localised microinjections of clonidine into the lateral reticular nucleus (which also includes the vasopressor C_1 nuclei of the RVLM) resulted in hypotension plus bradycardia and suggests that the C_1 nuclei may participate in the central actions of clonidine (Bousquet et al, 1981). More recently, Head et al (1987b) investigated the cardiovascular role of the A_1 cell group in the CVLM of rabbits. Chronic lesions in this area attenuated the bradycardia but not hypotension produced by intracisternal clonidine administration.

Thus evidence exists, albeit indistinct, that clonidine in the medulla oblongata, evokes haemodynamic responses. Further characterisation of the cardiovascular effects of clonidine in this area is warrented and the present study compared the effects of microinjecting clonidine directly into the C_1 plus A_1 cell groups of the rostral and caudal ventrolateral medulla.

3.2 Methods

The animal studies described in this chapter were carried out using Wistar-Kyoto rats weighing between 250 - 320 g. Groups of 6 - 9 were used in all experiments.

3.2.1 Preparation of Animals for Intraparenchymal Microinjection Studies and Measurement of Arterial Pressure and Heart Rate

Anaesthesia was induced with a mixture of urethane (1 g kg^{-1}, i.p.) plus alpha-chloralose suspension (60 mgs kg^{-1}, i.p.) and maintained where necessary with urethane (300 mgs kg^{-1}, i.p.). The rats were placed in stereotaxic frame and vehicle or drug microinjected into either the caudal or rostral ventrolateral medulla using the methods and coordinates described in Section 2.3. Arterial pressure and heart rate were measured from an arterial catheter inserted the previous day (Section 2.2). At the end of each experiment the site of injection was confirmed by histological examination (Section 2.4).

3.2.2 Statistical Analysis

Changes in mean arterial pressure and heart rate from basal values are denoted ΔMAP and ΔHR. All values are quoted as means $\pm$ s.e.mean. The significant changes in MAP and HR from control values following drug injections were determined by Repeated Measures Analysis of Variance, with a bonferoni correction included where appropriate for multiple comparisons.

3.3 Results

3.3.1 Control Responses in the Caudal and Rostral Ventrolateral Medulla

No significant effect on MAP or HR was observed after the

microinjection of saline into the caudal (CVLM) or rostral (RVLM) ventrolateral medulla of anaesthetised rats (Figure 3.1 and Figure 3.2). Furthermore injection of neuropeptide Y (NPY) or clonidine into nearby control areas lateral or dorsal to these regions did not produce any significant haemodynamic response.

3.3.2 Effect of Neuropeptide Y Microinjected into the Caudal Ventrolateral Medulla

The haemodynamic profile obtained after microinjection of NPY (25 and 50 pmol) into the CVLM is shown in Figure 3.1. Both doses of NPY microinjected into this area produced a fall in MAP. This hypotensive response reached statistical significance, with the higher dose of NPY (50 pmol), after five minutes. The maximum decrease in MAP was -7.4 $\pm$ 2.1 mmHg and -12.3 $\pm$ 3.0 mmHg, which occurred at 30 minutes post-injection in response to 25 and 50 pmol NPY respectively. The MAP returned to control values by one hour post-injection. The fall in HR elicited after injection of NPY into the CVLM, which was similar for both doses, was significantly different from control value for both doses at 30 minutes and persisted for one hour. Nadir was reached at 45 minutes and was -91.1 $\pm$ 21.5 bpm and -94.4 $\pm$ 28.1 bpm for NPY 25 and 50 pmol, respectively.

3.3.3 Effect of Neuropeptide Y Microinjected into the Rostral Ventrolateral Medulla

In the RVLM, neither concentration of NPY investigated (25 and 50 pmol) significantly influenced blood pressure or heart rate when compared to saline control (Figure 3.2).

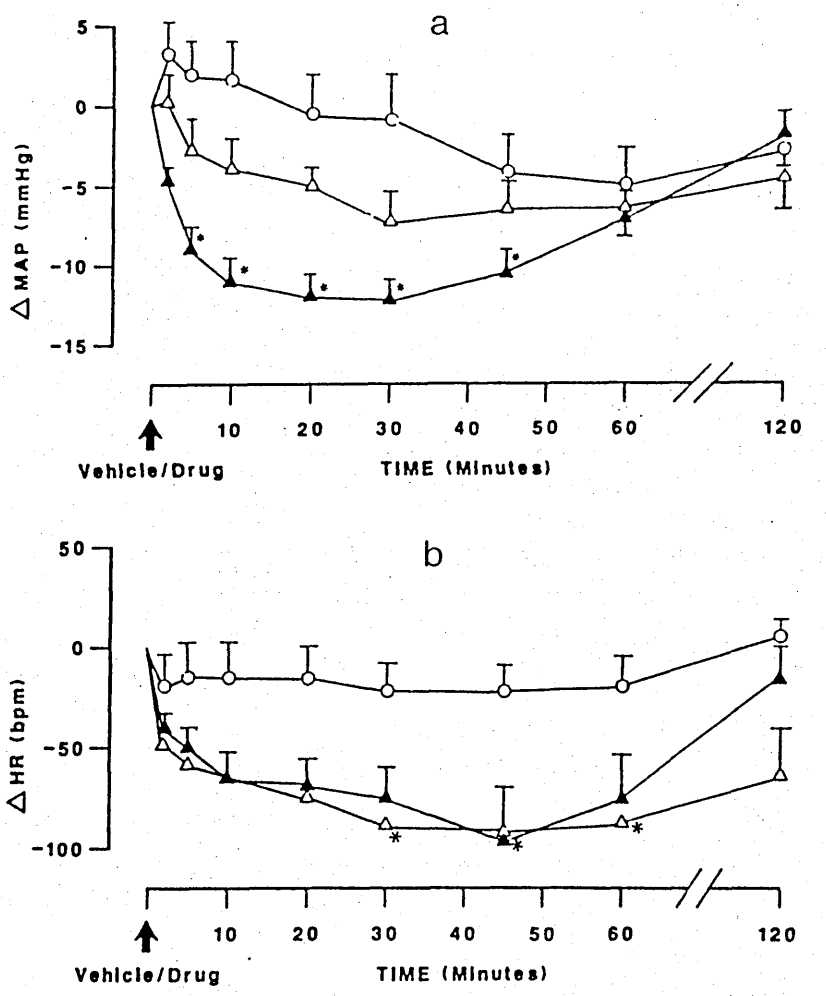


Figure 3.1

Effect of neuropeptide Y (NPY) microinjections into the caudal ventrolateral medulla on (a) mean arterial pressure (MAP) and (b) heart rate (HR).

o saline
Δ NPY (25 pmol)
▲ NPY (50 pmol)

Points represent mean changes from preinjection value; vertical bars show s.e.mean; * significant differences from saline control ($p < 0.05$).

Preinjection values (means + s.e.mean)	MAP (mmHg)	HR (bpm)
Saline; n = 6	96.1 ± 2.6	369.0 ± 14.1
25 pmol NPY; n = 9	101.0 ± 1.9	338.0 ± 11.3
50 pmol NPY; n = 9	102.0 ± 2.7	368.0 ± 4.2

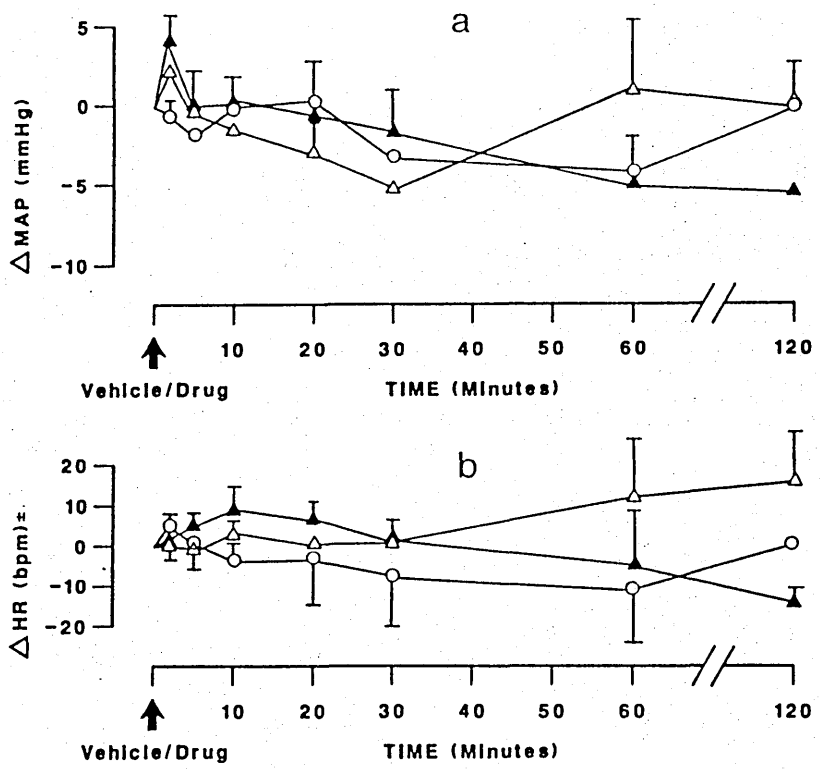


Figure 3.2

Effect of neuropeptide Y (NPY) microinjections into the rostral ventrolateral medulla on (a) mean arterial pressure (MAP) and (b) heart rate (HR).

○ Saline
△ NPY (25 pmol)
▲ NPY (50 pmol)

Points represent mean changes from preinjection value; vertical bars show s.e.mean;

Preinjection values (means ± s.e.mean)	MAP (mmHg)	HR (bpm)
Saline; n = 6	89.2 ± 3.3	368.8 ± 18.3
25 pmol NPY; n = 7	97.9 ± 4.9	382.8 ± 18.4
50 pmol NPY; n = 6	90.0 ± 2.6	360.0 ± 13.9

3.3.4 Effect of Clonidine Microinjected into the Caudal Ventrolateral Medulla

Clonidine (10 and 20 nmol) evoked a significant dose-related fall in MAP when microinjected into the CVLM (Figure 3.3). The maximum hypotensive effect following injection of clonidine 10 and 20 nmol (-21.0 ± 4.6 mmHg; -40.8 ± 6.5 mmHg, respectively) was manifest between 45 and 60 minutes and the reduction sustained for 2-3 hours. The bradycardia produced by clonidine resembled the response after injection of NPY in this region (Figure 3.3), with both concentrations of clonidine producing comparable falls in HR. The bradycardia reached the nadir at 45 minutes with a time course commensurate with the blood pressure response.

3.3.5 Effect of Clonidine Microinjected into the Rostral Ventrolateral Medulla

Clonidine (10 and 20 nmol) produced dose-related hypotension and bradycardia following microinjection into the RVLM (Figure 3.4). The peak hypotensive response of -21.6 ± 5.9 mmHg (10 nmol) and -36.0 ± 3.5 mmHg (20 nmol) was observed 30 minutes post-injection, with the fall in MAP lasting 2-3 hours at the highest dose. In contrast to the effect of clonidine on the HR response in the more caudal region (Section 3.3.4), the bradycardia induced in this area was dose-related. A similar time-course was demonstrated for the bradycardia, compared with the hypotensive response. The maximum fall in HR was - 54.6 ± 12.2 bpm and -110 ± 16.2 bpm following injection of

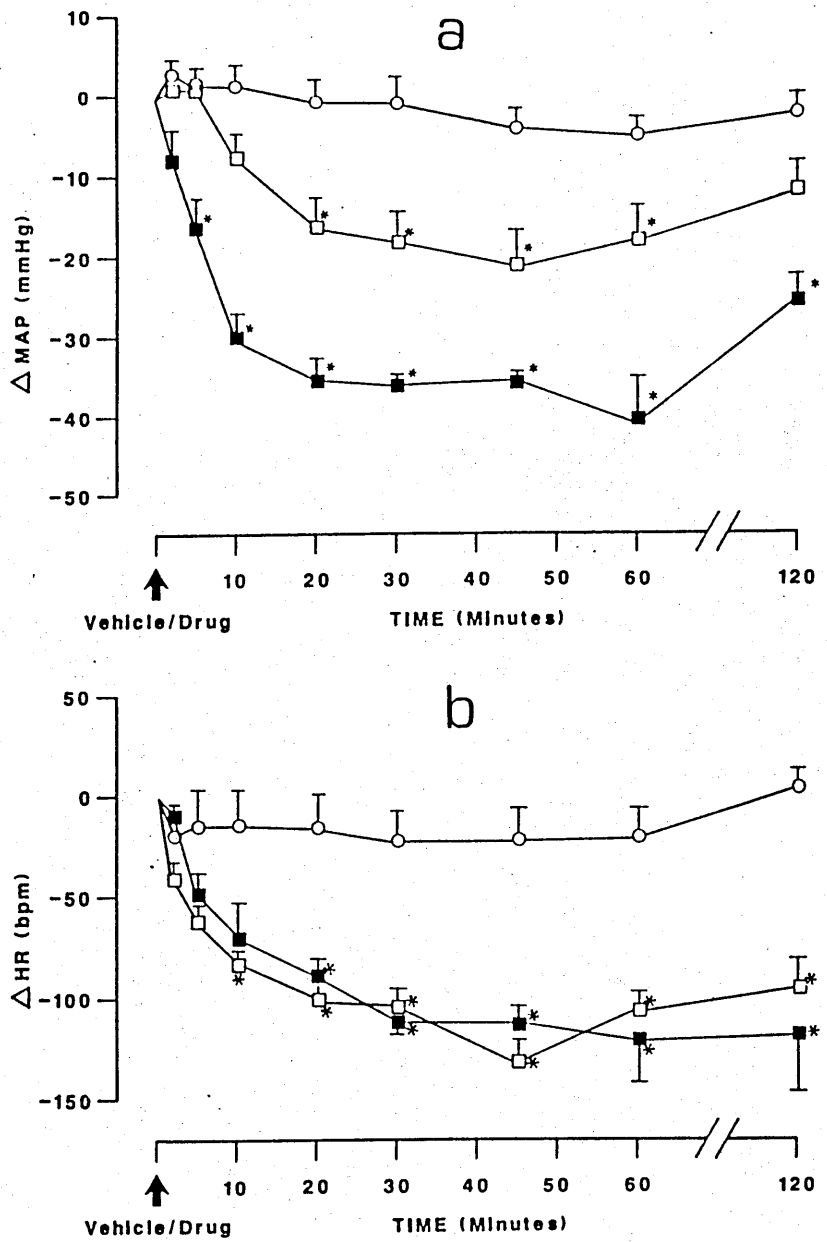


Figure 3.3

Effect of clonidine microinjections into the caudal ventrolateral medulla on (a) mean arterial pressure (MAP) and (b) heart rate (HR).

○ Saline
□ Clonidine (10 nmol)
■ Clonidine (20 nmol)

Points represent mean changes from preinjections values; vertical bars show s.e.mean; * significant differences from saline control ($p < 0.05$).

Preinjection values (means + s.e.mean)	MAP (mmHg)	HR (bpm)
Saline; n = 6	96.1 ± 2.6	369.0 ± 14.1
10 nmol Clonidine; n = 10	96.8 ± 2.5	373.0 ± 8.6
20 nmol Clonidine; n = 5	94.4 ± 1.7	339.0 ± 13.3

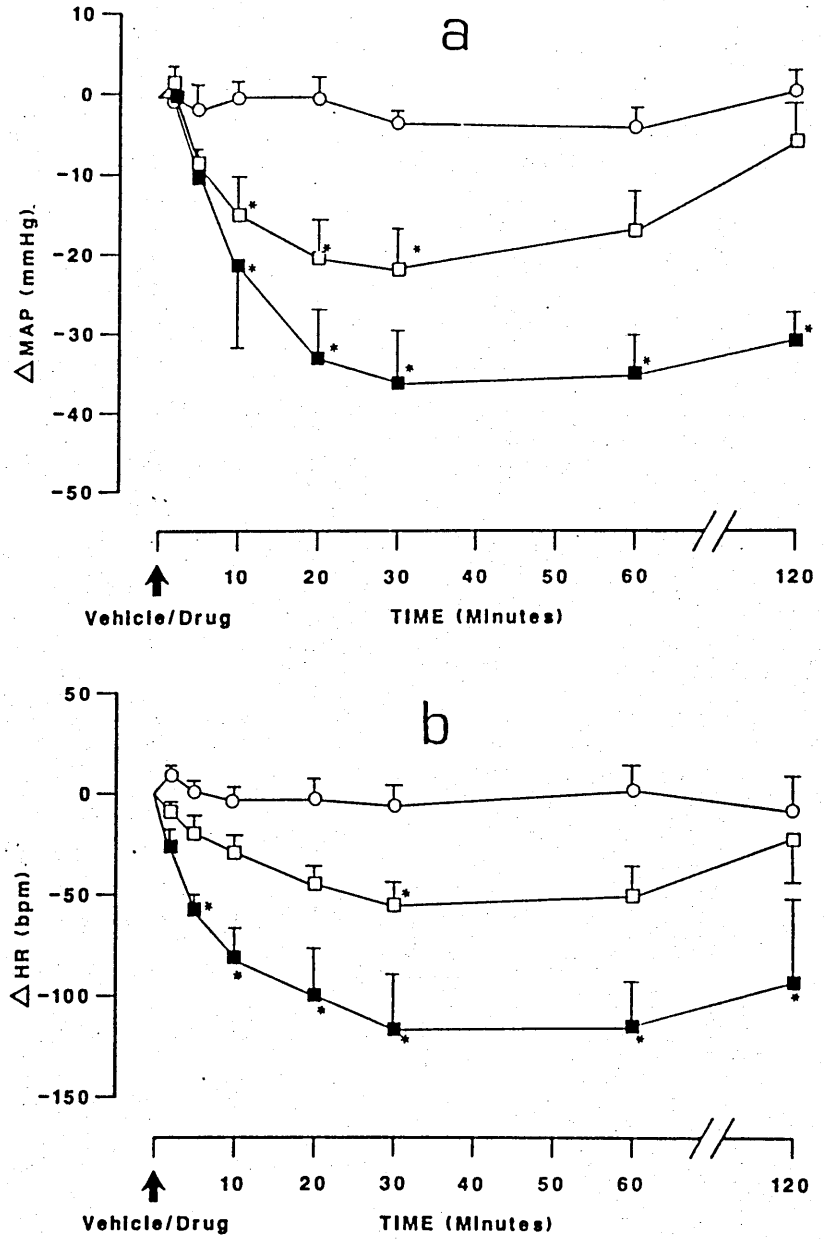


Figure 3.4

Effect of clonidine microinjections into the rostral ventrolateral medulla on (a) mean arterial pressure (MAP) and (b) heart rate (HR).

○ Saline
□ Clonidine (10 nmol)
■ Clonidine (20 nmol)

Points represent mean changes from preinjection values, vertical bars show s.e.mean; * significant differences from saline control ($p < 0.05$).

Preinjection values (means + s.e.mean)	MAP (mmHg)	HR (bpm)
Saline; n = 6	81.1 ± 3.3	368.3 ± 18.3
10 nmol Clonidine; n = 8	97.3 ± 1.9	375.0 ± 15.9
20 nmol Clonidine; n = 5	88·0 ± 6.0	347.0 ± 29.1

clonidine 10 and 20 nmol, respectively.

3.4 Discussion

3.4.1 Effects of Neuropeptide Y in the Ventrolateral Medulla

Using a discrete microinjection technique the present study demonstrates that NPY evokes cardiovascular effects following injection into a specific region of the ventrolateral medulla. In the CVLM, injection of NPY results in a dose-related hypotension which is analogous with the peptide's effect after intracisternal administration (Macrae and Reid, 1988). The haemodynamic response in the present study most likely involved the group of noradrenergic (A_1) neurones located in this caudal area (Dahlstrom et al, 1964). The mechanism through which the A_1 neurones alter arterial pressure is not clear at present. However, these cells do not appear to project to the spinal cord (Ross et al, 1981a). An inhibitory pathway from the A_1 cells to the vasopressor (C_1) cells of the RVLM, which do project directly to the spinal cord, has been implicated, however as discussed previously in Section 1.3.5 other pathways may contribute.

Further evidence for an involvement of the sympathetic nervous system in the actions of NPY in the CVLM arises from experiments carried out by Willette et al (1987). They report that chemical excitation of the CVLM in anaesthetised rats using the excitatory amino acid L-glutamate, results in a reduction of regional vascular resistance via an alteration of sympathetic nerve activity. These data together with the localisation of

NPY-immunoreactive terminals around the A_1 nucleus (Harfstrand et al, 1987a) suggest that NPY in the CVLM stimulates the A_1 neurones to evoke a reduction in sympathetic outflow and produce a fall in blood pressure.

Although NPY elicits a pronounced vasodepressor response in the CVLM, no effect was observed after NPY administration in the more rostral area. Harfstrand et al (1987a) have shown a low to moderate density of NPY-like immunoreactive terminals in the RVLM, suggesting the presence of endogenous NPY in this area. Therefore the lack of effect of exogenously administered NPY is curious. Furthermore, the importance of the area, which includes the C_1 adrenergic neurones, in tonic and reflex cardiovascular regulation is well established. Ross et al (1984b) have shown that electrical stimulation of the C_1 area results in significant increases in arterial pressure, while electricl or chemical lesion of these neurones cause a profound fall in arterial pressure (Dampney et al, 1982).

The haemodynamic effects of NPY have been investigated in another discrete area of the medulla, the NTS (Carter et al, 1985). In this region the direction of the blood pressure response to NPY was dose-dependent, 470 fmol induced a pressor response, 4.7 pmol a fall in blood pressure and the low dose of 47 fmol was ineffective. Therefore it is possible that an effect of NPY in the RVLM would be observed by extending the dose-range. However, this is unlikely since the doses used in the present study (NPY; 25 and 50 pmol) are similar to the dose producing hypotension in the NTS.

Recent evidence indicates that subtypes of receptors for NPY may also exist in the central as well as the peripheral nervous systems and this may explain the different actions of NPY demonstrated in the CVLM and RVLM. Initially it was proposed by Wahlestedt et al (1986), that two distinct subtypes of NPY receptors were responsible for the pre- and post- junctional actions of NPY in the peripheral sympathetic neuroeffector junction. This hypothesis was based on the ability to mimic the effects of NPY at the presynaptic but not postsynaptic receptor with the C-terminal fragment of NPY_{13-36} (Section 1.5.4). Sheikh et al (1989), using monoiodinated radioligands of both the intact NPY molecule and the C-terminal fragment NPY_{13-36} in human and porcine brain, has apparently characterised two subtypes of NPY binding sites. The Y_1 subtype was identified on a human neuroblastoma cell line and binds NPY with a dissociation constant in the nanomolar range but does not bind NPY_{13-36}. In agreement with Walker and Miller (1988) who utilised rat forebrain membranes, Sheikh et al (1989), also defined a second binding site (Y_2) which was in the majority, and bound NPY with a high affinity plus the C-terminal fragment NPY_{13-36}. A functional role of these binding sites was considered by Fuxe et al (1987a) who observed in conscious rats, that intraventricular injection of NPY lowered blood pressure whereas NPY_{13-36} had no significant haemodynamic action. This indicates the possible involvement of the Y_1 receptor in cardiovascular control. Although it would appear from the limited data

available that the Y_2 subtype of NPY binding site does not directly participate in the central control of blood pressure, it is possible that this subtype is involved in the central presynaptic actions of NPY which include the inhibition of neurotransmitter release from rat sensory neurones (Walker et al, 1988).

It has not yet been possible to establish the distribution of the subtypes of NPY binding sites in the medulla, indeed Martel et al (1986) using radioligand and autoradiographical techniques observed only a single class of high-affinity sites in rat brain membrane preparations. Further work is therefore required to determine whether the above reported differences in affinity and specificity represent the expression of two different NPY receptor types or reflect two different states of a single receptor. The differential synaptic location of the NPY binding site(s) may regulate the response to exogenously administered NPY in a specific area and explain the lack of effect of NPY in the RVLM. Alternatively although NPY-immunoreactive terminals have been demonstrated in the rostral and caudal ventrolateral medulla (Harfstrand et al, 1987a), reports vary in the ability to determine the presence of NPY-binding sites in these regions (Nakajima et al, 1986; Martel et al, 1986). Mismatches between the distribution of NPY-like immunoreactive terminals and NPY binding sites exist throughout the central nervous system (Section 1.5.3). Thus the presence of the former may not always be a reliable indicator of a functional role for NPY in the region under investigation.

In addition to the hypotensive response, NPY administered into the CVLM elicited a fall in HR, which had a similar time course to the blood pressure response. These results are similar to those of Willette et al (1987), who observed that the vasodepressor response to chemical stimulation of the A_1 region in rats was accompanied by bradycardia. In the present study, in contrast to the effect on arterial pressure, the fall in HR after NPY microinjection into the CVLM was not dose-dependent. It is possible that the mechanisms involved in the regulation of heart rate are more sensitive to NPY than those of blood pressure. Thus although the blood pressure response to the lower dose of NPY was submaximal, the heart rate response may have been maximal. Moreover the nucleus ambiguus is in close proximity to the injection site. This area is the origin of most cardio-inhibitory vagal projections and may therefore be involved in the heart rate response. This area is included in a plexus of NPY-IR nerve terminals which surrounds the CVLM. Indeed, Macrae and Reid (1988), have demonstrated that the prolonged bradycardia elicited by microinjections of NPY into the CVLM is significantly reduced, but not abolished, in vagotomised rats. This incomplete attenuation of the heart rate response to NPY in vagotomised rats probably reflects the participation of cardiac sympathetic, as well as vagal pathways in the response.

The results of the present study confirms the role of NPY in the central regulation of cardiovascular control with the addition of the CVLM to the list of discrete regions where NPY

has been observed to produce a haemodynamic effect.

3.4.2 Effects of Clonidine in the Ventrolateral Medulla

A prolonged hypotension and bradycardia were demonstrated after microinjecting clonidine into the rostral and caudal regions of the ventrolateral medulla. In the CVLM, the fall in blood pressure resembled the response to electrical or chemical (glutamate) stimulation of this region, previously reported in rabbits (Blessing and Reis, 1982). The bradycardia produced by clonidine in the CVLM was not dose-related and in this respect resembled the heart rate response to NPY in the same area. As discussed previously (Section 3.4.1), the involvement of the nucleus ambiguus in the effects of NPY in the CVLM has been reported. Thus, the bradycardic response to clonidine may also be a result of diffusion of the peptide to the nucleus ambiguus or an action on neuronal pathways from the CVLM to nucleus (Macrae and Reid, 1988).

The dose-related reduction in blood pressure and heart rate observed following injection of clonidine into the RVLM, is consistent with an analogous microinjection studies carried out by Granata et al (1984; 1986), in anaesthetised and paralysed Sprague-Dawley rats.

A reduction in sympathetic nerve activity to the heart and blood vessels together with a facilitation of the vagal baroreceptor reflex was proposed to mediate the central cardiovascular actions of clonidine (Sun and Guyenet, 1986; Schmitt et al, 1967). More recently, Willette et al (1987),

observed that chemical stimulation of the neuronal pools in the CVLM and RVLM, using L-glutamate, resulted in a differential regulation of regional blood flow. Microinjection of L-glutamate into the CVLM produced a decrease in sympathetic nerve activity, blood pressure and heart rate, associated with a reduction in mesenteric and hindquarter vascular resistance. In contrast, the opposite effects were observed in the RVLM, namely an increase in sympathetic nerve activity, blood pressure and heart rate with a concomitant increase in the resistance of all vascular beds monitored. Since regulation of sympathetic outflow mediates the effects of L-glutamate stimulation in the RVLM and CVLM, it is possible that the effects of clonidine in these regions may involve a similar mechanism of action. However, the congrous effects of clonidine observed in the RVLM and CVLM in the present study, are in apparent conflict with the differing actions of L-glutamate, demonstrated in both areas by Willette and colleagues. It is therefore necessary to find an alternative explanation in order to understand this anomaly.

Controversy exists concerning the possible interference of anaesthesia in the haemodynamic responses of clonidine. Vlahakos et al (1985) reported that in conscious animals clonidine injected into the nucleus tractus solitarius of the medulla produced pressor responses instead of the reported depressor responses observed in anaesthetised animals (Gurtu et al, 1982). Thus it was suggested that central forebrain pressor effects of clonidine are attenuated in the anaesthetised rat. However Punnen et al (1987) were unable to confirm these observations

since the hypotensive action of clonidine in the ventrolateral medulla was qualitively identical in the conscious mid-collicular decerebrate rats and rats anaesthetised with pentobarbital or urethane. In the present study urethane was used to provide a prolonged and stable anaesthesia since, at least in the latter study, it did not appear to alter the cardiovascular response to clonidine.

Although the antihypertensive actions of clonidine have been considered to be due to an action at central α_2 adrenoceptors, the synaptic location of these receptors has not been clearly established. Unnerstall et al (1984) demonstrated that the distribution of α_2 adrenoceptors in the brainstem correlates well with the location of central noradrenergic and adrenergic neurones. A post synaptic site of action for clonidine has been suggested by Haeusler (1974). This author observed that clonidine was still able to reduce sympathetic outflow following depletion of central noradrenaline stores with reserpine plus α-methyl-p-tyrosine implying that a tonic release of noradrenaline is not a prerequisite for the hypotensive action of clonidine (Starke and Montel, 1973). In addition, prior treatment with 6-hydroxydopamine, to destroy noradrenergic nerve terminals in the hindbrain and spinal cord, showed that clonidine was still hypotensive although bradycardia was attenuated indicating the involvement of noradrenergic terminals at least in the latter action of clonidine (Korner and Head, 1983; Connor and Finch, 1981). Ligand binding studies have further revealed that

pretreatment with 6-hydroxydopamine does not alter the binding of (^{3}H) clonidine in the central nervous tissue (U'Prichard et al, 1977). Indeed substantial evidence exists to show clonidine-mediated lowering of blood pressure by an action at postsynaptic receptors and may explain the mechanism by which clonidine lowers blood pressure in the CVLM (Kobinger, 1978; Bousquet and Schwartz, 1983).

Nevertheless a presynaptic mechanism in which clonidine inhibits the release of adrenaline has been proposed (Scatton et al, 1979; Atkinson et al, 1984). This is supported by the indirect evidence that adrenergic neurones are resistant to the neurotoxic effects of 6-hydroxydopamine administration and that pretreatment with this agent does not reduce the hypotensive actions of intracisternal clonidine (Reid et al, 1976). Thus, the hypotensive action of clonidine shown in the vasopressor RVLM may be a consequence of a presynaptic action on the adrenergic terminals of colaterals, or interneurones in this region, rather than a direct postsynaptic effect. This would result in the inhibition of transmitter release, leading to a reduction in the activity of the vasopressor neurones and subsequent hypotension. Consistent with this hypothesis is the observation that the blood pressure lowering effect of clonidine is accompanied by a decreased capacity to synthesise adrenaline in the ventrolateral medulla (Atkinson et al, 1986). Moreover, Ward-Routledge et al (1988) demonstrated that intracisternal administration of idazoxan, the α_2-adrenoceptor antagonist, enhanced the pressor effects of C_1 stimulation supporting the existence of a α_2-

autoreceptor involved in blood pressure control. Local brainstem networks containing gamma-amino butyric acid, glutamate and acetylcholine can modify the actions of ventrolateral medullary neurones and may also account for the similar actions of clonidine in both regions (Section 1.3.4).

Recently, Bousquet et al (1984) showed that imidazoles, such as cirazoline which lack α_2 adrenoceptor selectivity, are hypotensive when applied to the ventrolateral medulla Thus the hypotensive action of these agents, and even clonidine itself, may be effected through binding at non α_2-adrenergic receptors in this area. This has been confirmed by Ernsberger et al (1986), who have shown, using radioligand binding studies, that clonidine binds with high affinity not only to α_2-adrenergic receptors but also to a population of imidazoline binding sites in the ventrolateral medulla. The natural ligand for the imidazole receptors is unknown. One possibility is an endogenous clonidine displacing substance isolated from bovine brain (Atlas and Burstein, 1984). Like clonidine, this substance binds both to α_2-adrenergic and imidazole receptors in the VLM (Ernsberger et al, 1986) and produces hypotension when injected into the RVLM of the rat (Meeley et al, 1986). Thus the hypotensive action of clonidine in both areas studied may in part be mediated through these imidazoline receptors.

To summarise, a fall in blood pressure and heart rate was demonstrated after NPY and clonidine administration in the CVLM and for clonidine alone in the RVLM. Further characterisation in

terms of neuronal pathways and localisation of terminals and receptors in the ventrolateral medulla is necessary to resolve the apparent discrepancies and elucidate the action of NPY and clonidine in this region. The definition of the postulated receptor populations will be facilitated by the development of receptor isolation and purification techniques, as well as more specific ligands for these receptor subtypes.

CHAPTER FOUR

COADMINISTRATION OF NEUROPEPTIDE Y AND CLONIDINE IN THE VENTROLATERAL MEDULLA

Chapter Four

Coadministration of Neuropeptide Y and Clonidine in the Ventrolateral Medulla

4.1 Introduction

The colocalisation of neuropeptide-Y (NPY) with catecholamines in central nuclei associated with cardiovascular control implicates this peptide in the modulation of catecholamine pathways and haemodynamic regulation (Hokfelt et al, 1983a; Everitt et al, 1984). It has previously been shown that clonidine, the α_2-adrenoceptor agonist, as well as adrenaline and noradrenaline reduce blood pressure following central administration, most likely by an action on central adrenoceptors (Section 3.3; Zandberg et al, 1979; Kubo and Misu, 1981). Injection of NPY into similar areas also produces a hypotensive effect (Section 3.3). The similarity of the actions of catecholamines and NPY suggest that the coexisting peptide may cooperate with the endogenous transmitter(s) in blood pressure regulation. The possibility of central functional interactions between catecholamines and NPY has been further encouraged by reports of an NPY-mediated increase in dopamine turnover in the rat striatum and a reduction in noradrenaline turnover in the rat brainstem subsequent to the central injection of NPY (Flint-Beal et al, 1986; Vallejo et al, 1987).

More direct evidence of a central interaction between adrenoceptors and NPY binding sites derives from a series of quantitative autoradiographical experiments by Harfstrand et al

(1989). They reported that NPY in vitro or after intraventricular administration in conscious rats significantly reduced the density of α_2-adrenergic binding sites (using tritiated para-amino clonidine as a radioligand) in the medulla oblongata. In the same area, comparative studies revealed a decrease in the binding of iodinated NPY in the presence of in vitro or in vivo clonidine (Fuxe et al, 1986; Harfstrand et al, 1989). These findings indicate the existence of a receptor-receptor interaction and support earlier observations, using radioligand binding techniques, that NPY in vitro can alter the binding characteristics of α_2-adrenergic receptors. Using membrane preparations from the medulla oblongata, Agnati et al (1983) previously observed an increased number of α_2-adrenergic binding sites in the presence of NPY. In subsequent experiments however, the only consistent observation in this area was an NPY-evoked reduction in the affinity of the α_2-adrenergic binding sites for tritiated para-amino clonidine (Fuxe et al, 1986). Alteration of experimental conditions and concentrations of radioligands used between studies may account for these discrepancies.

Thus evidence of a reciprocal modulation of α_2-adrenoceptors and NPY binding sites exists in the medulla and it is proposed that this interaction may operate physiologically. In agreement with this view, Harfstrand et al (1984) demonstrated that simultaneous intracisternal injection of clonidine and NPY in anaesthetised rats does not lead to any additional lowering of blood pressure compared to that seen with

either drug alone, implicating a negative interaction between these receptors. Furthermore an inhibitory regulation was observed by Harfstrand and Fuxe (1987) who found that intraventricular coadministration of equi-potent doses of adrenaline and NPY significantly counteract the hypotensive effects of one another in conscious rats.

Based on the haemodynamic responses obtained for the individual microinjections of NPY and clonidine into the ventrolateral medulla presented in the preceding section (Section 3.3), the present study explored the prospect of a functional interaction between NPY and α_2-adrenoceptors in the same regions. Thus in the caudal (CVLM) and rostral (RVLM) ventrolateral medulla, the response to simultaneous microinjection of NPY and clonidine was investigated.

4.2 Methods

4.2.1 Coadministration of Neuropeptide Y and Clonidine in the Rostral and Caudal Ventrolateral Medulla

Groups of 6 - 12 male Wistar-Kyoto rats (250 - 320 g), anaesthetised with a combination of urethane and α-chloralose suspension (Section 2.2) were utilised for the studies contained within this chapter. The animals were prepared and unilateral microinjections into the RVLM or CVLM implemented as described in Section 2.3. A submaximal concentration of clonidine (10 nmol) and NPY (25 or 50 pmol) was co-administered in a total volume of 0.1 μl, over a time period of 20 seconds, consistent with the

individual injections of each agent.

4.2.2 Statistics

Analysis was carried out on the changes in mean arterial pressure (ΔMAP) and heart rate (ΔHR). The statistical test used - Repeated Measures Analysis of Variance - compared interval estimates for the change in response following coadministration of NPY and clonidine with the interval estimates for the sum of the individual changes in response reported in the previous chapter (Section 3.3). * $P < 0.05$ represents significant difference. All results are expressed as mean $\pm$ s.e.mean.

4.3 Results

4.3.1 Effect of Neuropeptide Y and Clonidine Co-administration in the Caudal Ventrolateral Medulla

The hypotensive effect produced by clonidine (10 nmol) in the CVLM was not altered by simultaneous injection of NPY (25 pmol), which alone had no significant effect on blood pressure (Figure 4.1). However, the bradycardia observed following the dual injection of NPY (25 pmol) and clonidine (10 nmol) was significantly less than the sum of the individual heart rate responses between 20 and 120 minutes (Figure 4.1).

Co-administration of clonidine (10 nmol) and the higher dose of NPY (50 pmol) evoked a fall in blood pressure which was similar in the time course and magnitude to the sum of their individual responses (Figure 4.2). The associated reduction in heart rate following this dual injection in the CVLM could also

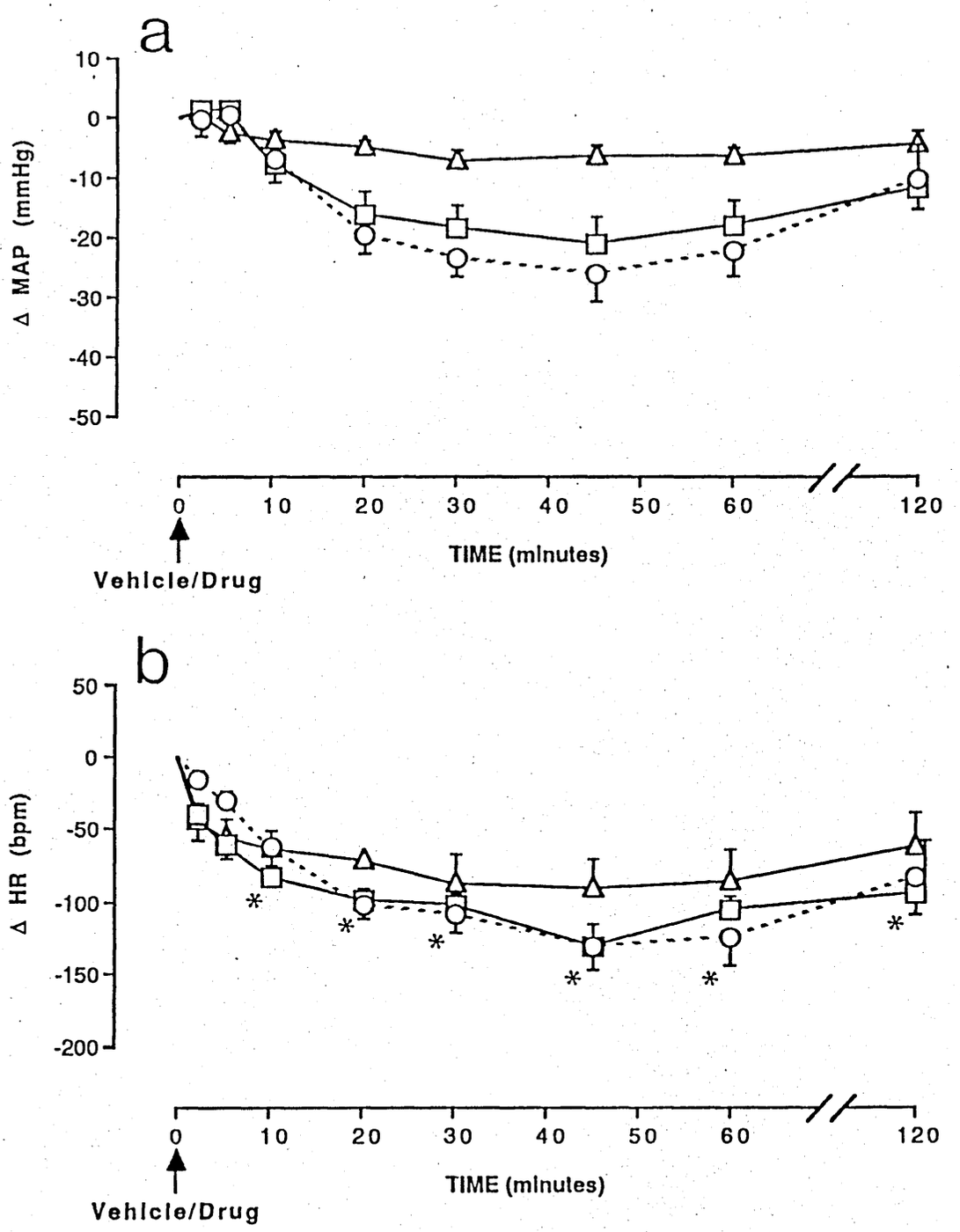


Figure 4.1

Effect of coadministration of neuropeptide Y (NPY) together with clonodine into the caudal ventrolateral medulla on (a) mean arteries pressure (MAP) and (b) heart rate (HR).

△ NPY (25 pmol)
□ Clonidine (10 nmol)
○ NPY (25 pmol) + clonidine (10 nmol)

Points represent mean changes from preinjection value; vertical bars show s.e.mean; * significant difference from sum of the individual values.

Preinjection values (means + s.e.mean)	MAP (mmHg)	HR (bpm)
25 pmol NPY; n = 9	101.0 ± 1.9	338.0 ± 11.3
10 nmol Clonidine; n = 10	96.8 ± 2.5	373.0 ± 8.6
Combination; n = 8	94.8 ± 2.9	376.0 ± 6.0

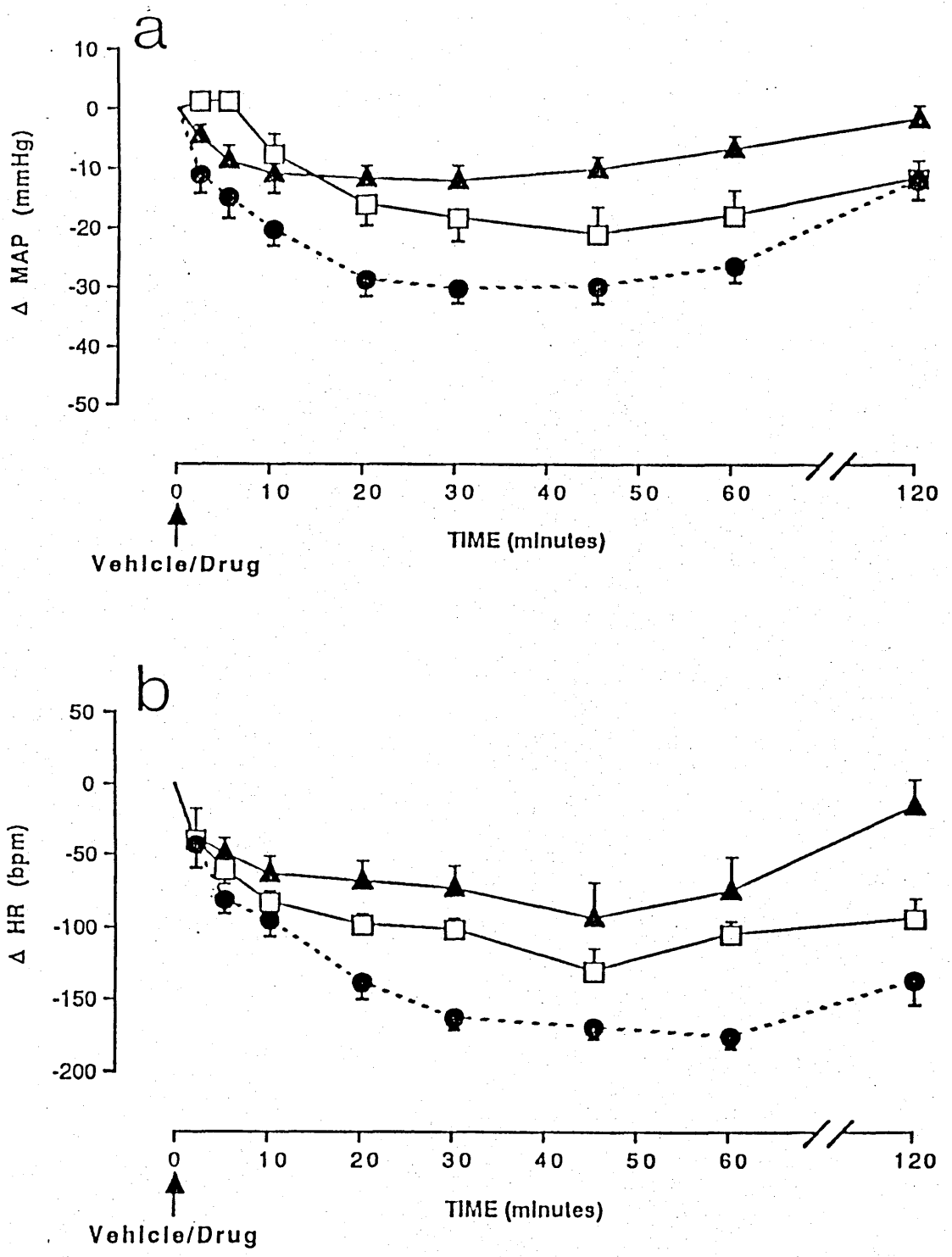


Figure 4.2

Effect of coadministration of neuropeptide Y (NPY) together with clonidine into the caudal ventrolateral medulla on (a) mean arterial pressure (MAP) and (b) heart rate (HR).

▲ NPY (50 pmol)
□ Clonidine (10 nmol)
● NPY (50 pmol) + clonidine (10 nmol)

Points represent mean changes from preinjection value; vertical bars show s.e.mean.

Preinjection values (means ± s.e.mean)	MAP (mmHg)	HR (bpm)
50 pmol NPY; n = 9	102.0 ± 2.7	368.0 ± 9.2
10 nmol Clonidine; n = 10	96.8 ± 2.5	373.0 ± 8.6
Combination; n = 8	94.8 ± 2.9	376.0 ± 6.0

be considered additive (Figure 4.2).

4.3.2 Effect of Neuropeptide-Y and Clonidine Co-administration in the Rostral Ventrolateral Medulla

Although NPY (25 and 50 pmol) alone produced no significant haemodynamic effect in the RVLM, it is still possible that the peptide could modulate the response to other agents. Therefore the cardiovascular changes induced by simultaneous injection of NPY (25 pmol) and clonidine (10 nmol) in the RVLM were investigated. The blood pressure and heart rate response to the co-administered NPY (25 pmol) and clonidine (10 nmol) had a similar profile and was not significantly different from the response to clonidine alone in the RVLM (Figure 4.3).

4.4 Discussion

The current study demonstrates that in the CVLM of anaesthetised rats, the reduction in blood pressure produced by coadministration of clonidine and NPY was not significantly different from the sum of their individual actions. Furthermore in the RVLM NPY (25 pmol), which alone had no significant effect on blood pressure, also failed to modify the hypotensive action of clonidine. This lack of interaction between α_2-adrenoceptors and NPY-binding sites, with reference to blood pressure in the VLM, is contrary to a report by Carter et al (1985) who demonstrated that the hypotensive response to noradrenaline (20 nmol) injected into the NTS of anaesthetised rats, was significantly modified by prior or simultaneous injection of an ineffective dose of NPY (47 fmol).

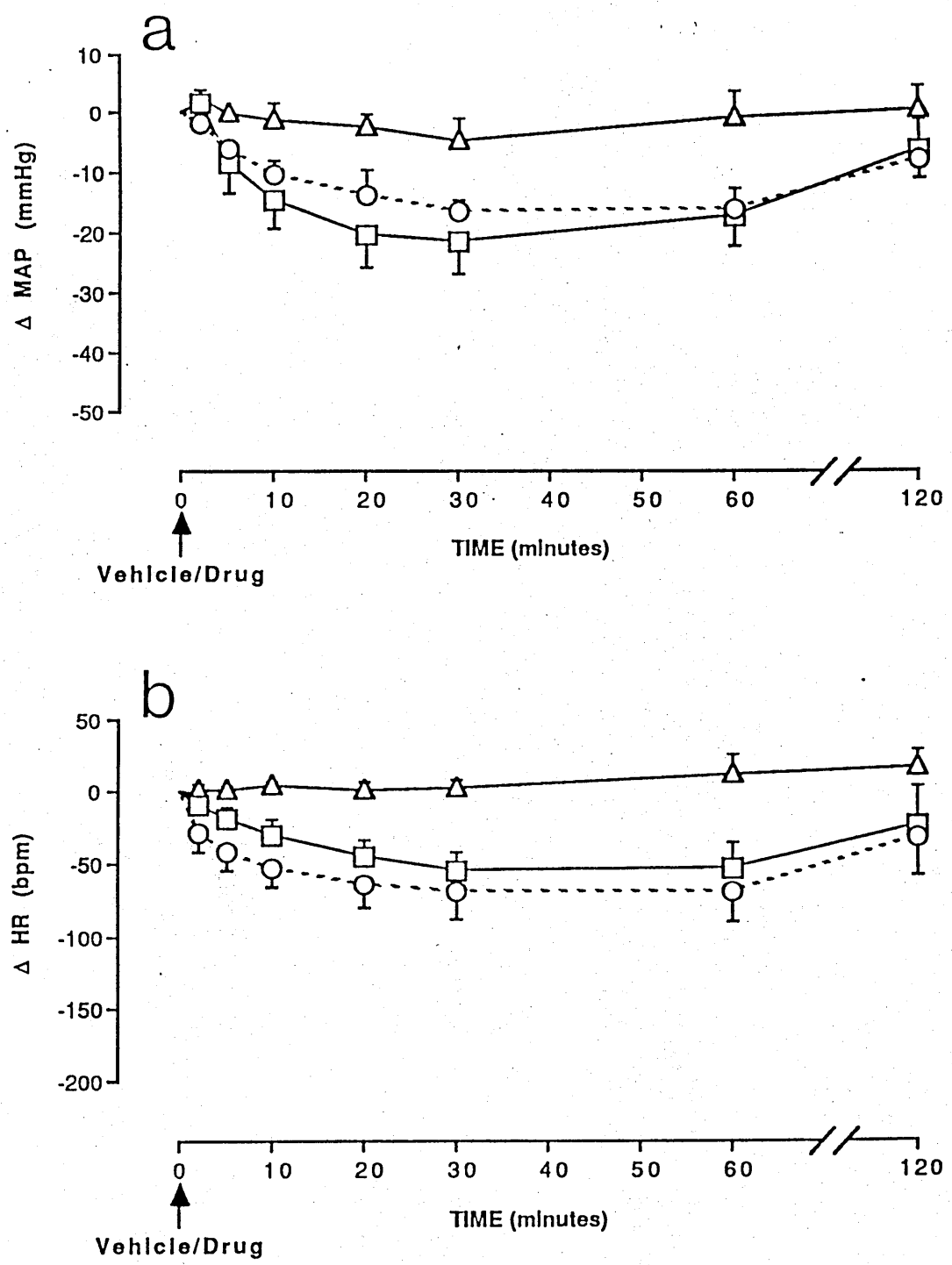


Figure 4.3

Effect of coadministration of neuropeptide Y (NPY) together with clonidine into the rostral ventrolateral medulla on (a) mean arterial pressure (MAP) and (b) heart rate (HR).

△ NPY (25 pmol)
□ Clonidine (10 nmol)
○ NPY (25 pmol) + clonidine (10 nmol)

Points represent mean changes; vertical bars show s.e.mean, preinjection values ± s.e.mean are as follows:-

Preinjection values (means + s.e.mean)	MAP (mmHg)	HR (bpm)
25 pmol NPY; n = 7	97.9 ± 4.9	382.8
10 nmol Clonidine; n = 5	88.0 ± 6.0	347.0 ± 29.1
Combination; n = 6	91.5 ± 2.51	385.8 ± 11.1

In addition, the same authors also observed that in the locus coeruleus co-administration of NPY (which itself was inefficacious) also blocked the haemodynamic responses to noradrenaline and clonidine (Vallejo and Lightman, 1986b).

The reason for the disparity between the above reports and the current study is not clear since NPY-immunoreactivity has been shown to be co-localised with catecholamines in the cell bodies and terminals of all the regions described (Hokfelt et al, 1983; Everitt et al, 1984; Harfstrand et al, 1987a). However, co-localisation of transmitters is not always indicative of a functional interaction. In the striatum, NPY and somatostatin are co-stored but have additive, rather than synergistic or antagonistic actions on dopamine turnover when co-injected, suggesting a separate mechanism of action (Kowall et al, 1985; Vincent and Johansson, 1983; Flint-Beal et al, 1986). Furthermore, an independent mechanism of action for NPY and clonidine has been proposed by Harfstrand et al (1984). They observed that prior intracisternal injection of RX 781094, an α_2-adrenoceptor antagonist, prevented the hypotensive actions of clonidine, but not the hypotensive action of NPY, indicating that NPY can exert its cardiovascular actions in the presence of α_2-adrenoceptor blockade. Indeed Vallejo and Lightman (1986a) found that intracerebroventricular administration of NPY still induced a pressor response after the depletion of central catecholamines by 6-hydroxydopamine. In contrast, the involvement of α_2-adrenoceptors in the response to NPY is supported by Chen et al

(1988) who demonstrated that intravenous pre-treatment with the α_2-adrenoceptor antagonist can prevent the hypotensive actions of intrathecal injections of NPY in the anaesthetised rat. Thus interactions between co-stored transmitters may be present in some central areas but cannot be considered ubiquitous.

Although the hypotensive response to clonidine in the CVLM was not altered by simultaneous injection of a low dose of NPY, combined injection of clonidine (10 nmol) and NPY (25 pmol) did produce a fall in heart rate which was significantly less than the sum of their individual heart rate responses. Therefore it appears that an antagonistic interaction exists between NPY-binding sites and α_2-adrenergic receptors, possibly linked to noradrenergic synapses in the CVLM associated with the control of heart rate. In agreement with this postulate, Fuxe et al (1986) also observed that the bradycardia evoked by the combined intracisternal administration of submaximal doses of NPY and clonidine was attenuated compared to the summed responses of each drug.

The participation of the peptide and catecholamines in the regulation of haemodynamic responses may occur at the post-synaptic site either at the level of the recognition site and/or the level of the coupling device (Fuxe et al, 1984; 1987b). More recently a presynaptic interaction has also been indicated. Martire et al (1986) demonstrated that NPY (1 nmol) enhanced the inhibitory effects of the α_2 agonist clonidine, on the potassium induced release of (^{3}H)-noradrenaline in synaptosomes from the rat medulla oblongata. This modulation of catecholamine turnover

has been corroborated by in vivo studies by Fuxe et al (1987b) where it was reported that intraventricular administration of NPY (7.5 pmol) reduced adrenaline release in the caudal dorsomedial medulla. Furthermore a tendency for an increased release of NPY was observed in the rostral dorsomedial medulla subsequent to intracisternally administered clonidine (3.75 nmol) in rats (Fuxe et al, 1987b). These data suggest a reciprocal presynaptic regulation exists between α_2 adrenoceptors and NPY receptors in this region. The relative importance of the presynaptic and post-synaptic receptor interactions in the medulla oblongata requires further investigation and may vary between regions or depend on the concentration of exogenous agent used (Harfstrand et al, 1987b).

In conclusion no apparent interaction was demonstrated for the combined administration of clonidine and neuropeptide Y with regard to the blood pressure response, in the ventrolateral medulla of anaesthetised rats. In the CVLM an antagnostic action on the heart rate response was observed following co-administration of clonidine and NPY. Further experiments are warranted to elucidate the exact mechanisms and possible subtypes of receptors involved in the modulatory response between clonidine and NPY illustrated here and previously reported in the medulla oblongata.

CHAPTER FIVE

EFFECT OF NEUROPEPTIDE Y ON cAMP LEVELS IN SLICES OF RAT MEDULLA OBLONGATA

Chapter Five

Effect of Neuropeptide Y on cAMP Levels in Slices of Rat Medulla Oblongata

5.1 Introduction

Despite the proposed functional role for neuropeptide Y (NPY) in the central nervous system, corroborated by the results presented in Section 3.3, the mechanism(s) of its haemodynamic action has not been clearly established. High affinity saturable binding sites for NPY have been characterised throughout the rat brain including the lower brainstem, using (^{125}I)-NPY as a radioligand (Unden et al, 1984; Nakajima et al, 1986; Harfstrand et al, 1986). The equilibrium binding of the NPY radioligands in the rat central nervous system has been shown to be altered by guanine nucleotides (Unden and Bartfai, 1984). Chang et al (1985) demonstrated that GppNHp (a stable analogue of GTP), GTP and GDP inhibited, in a concentration dependent manner, the specific binding of (^{125}I)-NPY to rat hippocampal membranes. A maximal decrease in NPY binding of approximately 50% of the control value was obtained in the presence of GppNHp (10^{-4}M). Moreover this inhibitory effect is not restricted to hippocampal membranes since a significant number of NPY binding sites, in the rat cerebral cortex and area postrema have also been reported to be sensitive to guanine nucleotides (Unden and Bartfai, 1984; Nakajima et al, 1986). These observations are of particular interest since guanine nucleotide binding proteins (G-proteins) have been implicated in the coupling of neurotransmitter or

hormone receptor complexes to intracellular second messenger systems (Section 1.4; Rodbell, 1980; Joseph, 1985). For example, guanine nucleotides have been shown to lower the binding affinity of agonists such as noradrenaline and acetylcholine in several systems (Kent et al, 1980; Berrie et al, 1979), as well as mediating the hormonal stimulation and inhibition of adenylate cyclase (Rodbell, 1980).

Recently it was reported that NPY inhibits forskolin-stimulated cAMP accumulation in feline cerebral blood vessels (Fredholm et al, 1985). This repressive action of NPY on the cAMP system was also later observed in membrane preparations of rat hippocampus and cortex (Petrenko et al, 1987; Westlind-Danielsson et al, 1987). In the former region, a GTP dependence for the NPY-attenuation of adenylate cyclase activity was also demonstrated, giving indirect evidence of the involvement of an inhibitory G-protein in the coupling of the NPY receptor complex to adenylate cyclase (Petrenko et al, 1987). The object of the present study therefore was three-fold. First to examine the effect of NPY on cAMP accumulation in slice preparations from rat medulla oblongata, an important area in the regulation of blood pressure (Section 1.3). Second, since other investigators have shown inhibitory effects of NPY on the cAMP system in the cortex using different techniques, the present study will also examine the effects of NPY on cAMP levels in the frontal cortex to allow comparison with those studies and with the proposed investigation of the effects of NPY in the medulla oblongata. Previous investigations have also observed that the inhibitory action of

NPY in other brain areas was best investigated in the presence of an activator of adenylate cyclase. Forskolin, a diterpene derivative as shown in Figure 5.1, which directly stimulates the catalytic subunit of the adenylate cyclase enzyme without involving the regulatory guanine binding proteins, was utilised for this purpose (Seamon et al, 1981). Assuming that NPY alters the levels of cAMP in the medulla oblongata, the third aim of the study was to examine the involvement of inhibitory guanine nucleotide binding proteins in the transduction mechanism associated with NPY-regulation of adenylate cyclase. Pertussis toxin (PTX) is known to uncouple the receptors for inhibitors of adenylate cyclase by ADP-ribosylation of a subunit of the inhibitory guanine nucleotide binding component of adenylate cyclase (Burns, 1988; Katada and Ui, 1982). Pretreatment with this toxin (intracisternal administration to concentrate the toxin around the medulla oblongata) would therefore attenuate or abolish the effect of inhibitory signals on adenylate cyclase. Thus, a more direct and specific approach was employed than observing the GTP-dependence of NPY on inhibition of cAMP levels (Petrenko et al, 1987), which would give insight into the mode of the hypotensive action of NPY in the medulla oblongata.

5.2 Methods

5.2.1 Preparation and Preincubation of Brain Slices

This procedure for the preparation and preliminary incubation of the medullary or cortical slices is described in

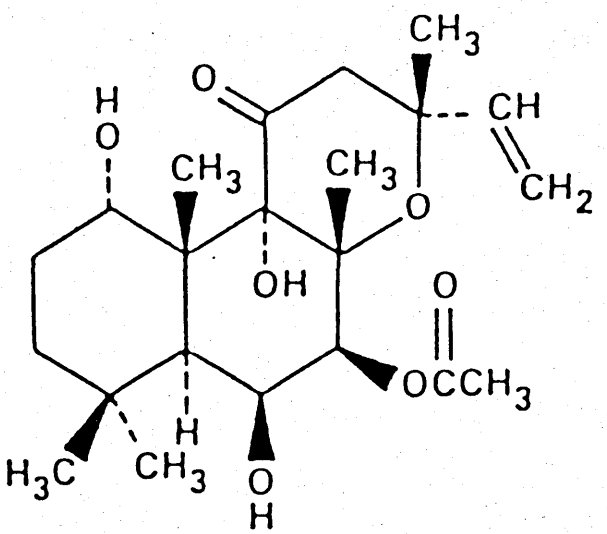


Figure 5.1

Structure of the diterpene forskolin

Section 2.8.1.

5.2.2 Drug Treatment of Slices

After the preliminary incubation stage, NPY or vehicle saline, in a volume of 20 µl was added to the appropriate tubes and incubated for five minutes. Forskolin or the vehicle ethanol (20 µl) was added after this stage and the tissue incubated for a further ten minutes (Figure 5.2). The reaction was terminated, tissue cAMP released and measured as detailed in Sections 2.8.2 and 2.8.3.

5.2.3 Pretreatment with Pertussin Toxin

Each animal was anaesthetised with methohexitone sodium (60 mgs kg^{-1}, i.p.) and an area of skin above the atlanto-occipital membrane shaved and cleaned with a sterile swab. The rat was then placed in a stereotaxic frame, with the head flexed approximately $45^{o}C$ down from the horizontal position. The atlanto-occpital membrane was located by touch and a 30-gauge needle connected to a length of polyvinyl tubing filled with sterile saline was implanted into the cisterna magna. Access to cerebrospinal fluid confirmed by the movement upwards and downwards of the miniscus when the column of fluid was lowered and raised in relation to the animal. The needle was left in position and a second length of tubing filled with either Pertussis Toxin or saline was connected. The toxin (2 µg; 5 µg) or vehicle saline (0.9% w/v) was then microinjected into the cisterna magna in a volume of 10 µl, using a Hamilton syringe, over a period of two minutes. Both groups of animals (control

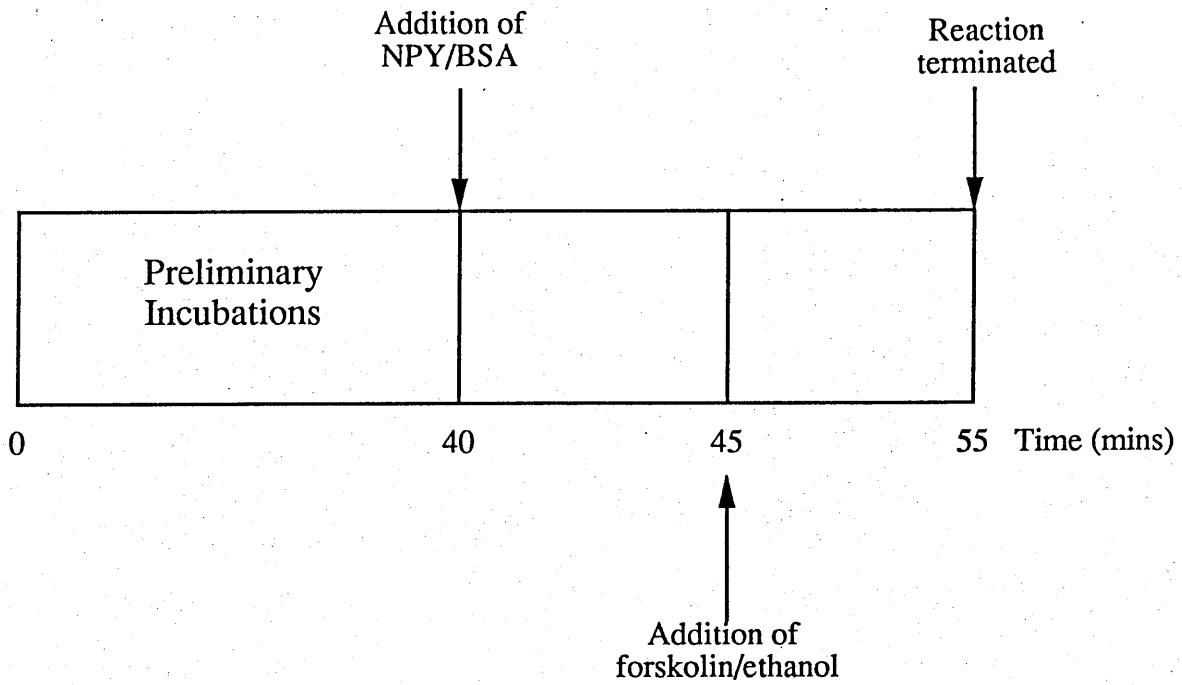


Figure 5.2

Protocol for the investigation of the effect of neuropeptide Y (NPY) on basal and forskolin-stimulated cAMP levels in slices of the rat medulla oblongata.

and PTX-treated) were allowed to recover for a period of 24 hours before continuing with the study.

5.2.4 Statistics

All values are expressed means ± s.e.mean. Statistical analysis was carried out using Repeated Measures Analysis of Variance (ANOVA) with the Bonferroni correction for multiple comparisons. * $P < 0.05$ denotes significant difference from basal values or as indicated by the bar.

5.3 Results

5.3.1 Effect of Neuropeptide Y on Basal and Forskolin-stimulated cAMP Accumulation in the Medulla Oblongata

Forskolin (10^{-5}M) significantly increased cAMP accumulation in the slice preparation of the medulla oblongata compared to control (basal) values, as demonstrated in Figure 5.3. The magnitude of the response to forskolin varied between experiments. NPY (10^{-7} - 10^{-5}M) alone, did not have any significant effect on the basal levels of cAMP. However NPY in the presence of forskolin appeared to be inhibitory with a significant attenuation of the forskolin-stimulated cAMP production observed at 10^{-6}M NPY.

5.3.2 Effect of Neuropeptide Y on basal and forskolin-stimulated cAMP accumulation in the cortex

In the frontal cortex NPY (10^{-6}M) again did not alter the basal levels of cAMP (Figure 5.4). Although these basal levels

MEDULLA OBLONGATA

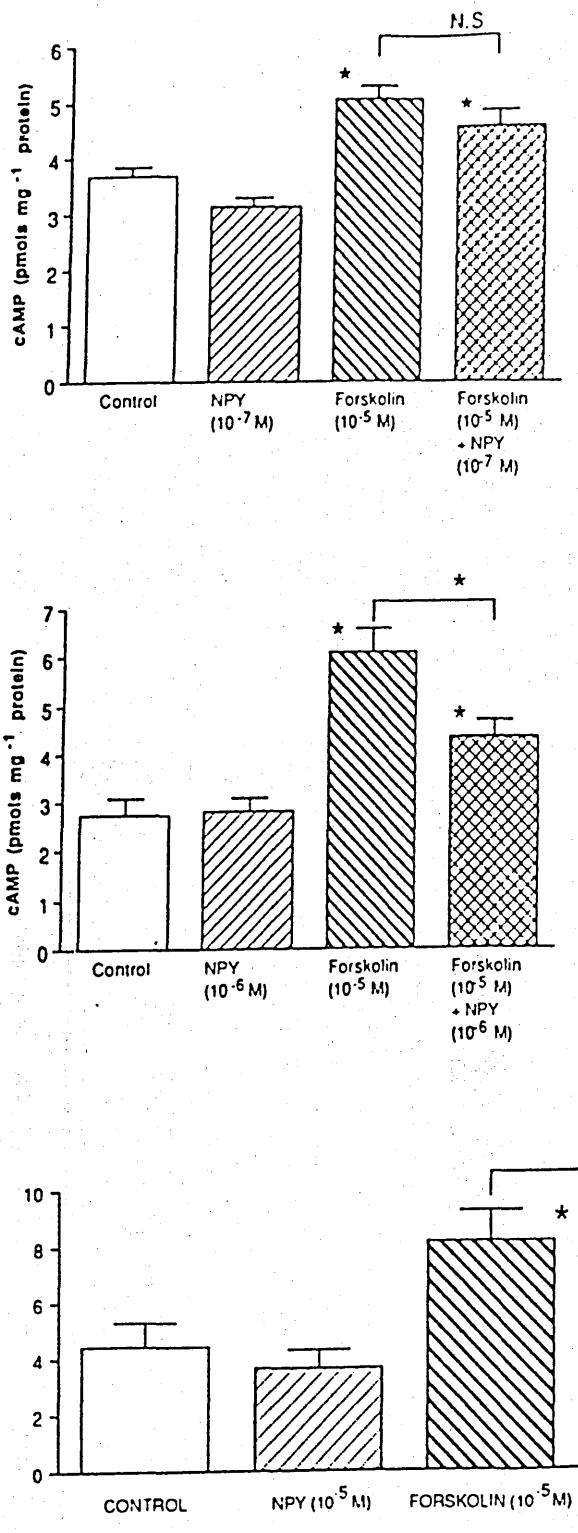


Figure 5.3

Effect of neuropeptide Y (NPY) on basal and forskolin-stimulated cAMP levels in the medulla oblongata.

Results are expressed as means ± s.e.mean, n = 4-7;
* significant difference from control (basal) value or as indicated by the bar.

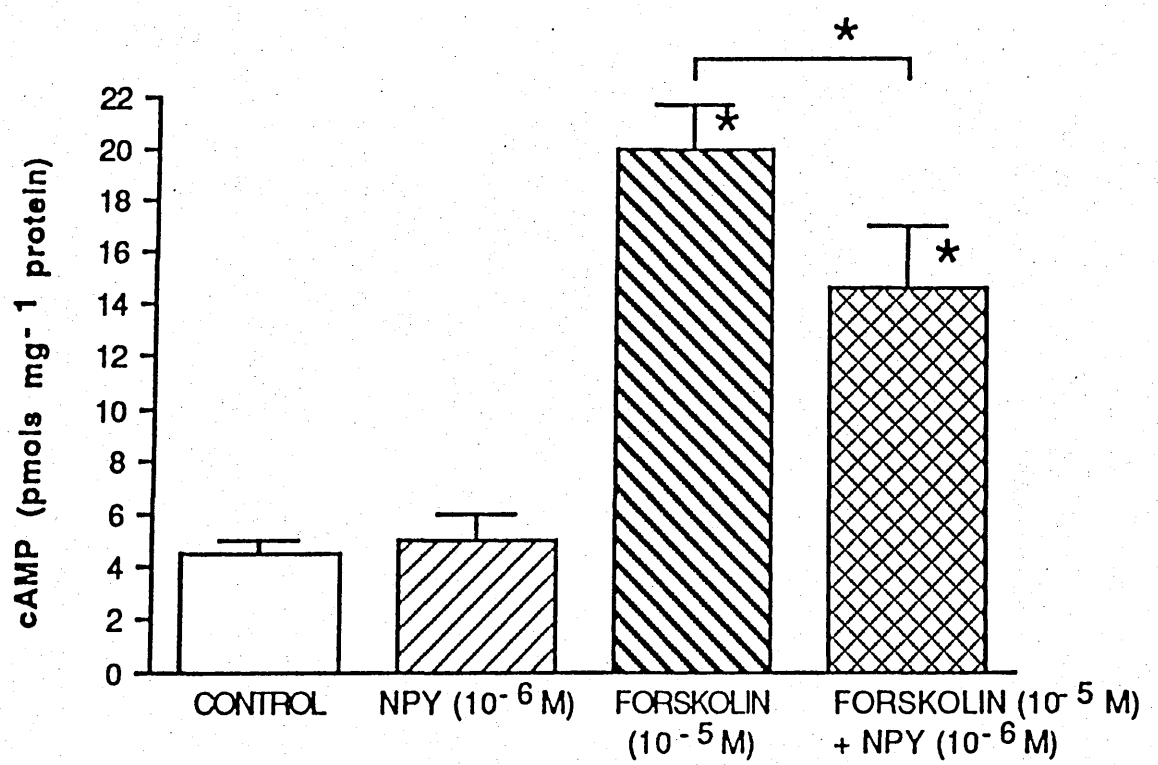


Figure 5.4

Effect of neuropeptide Y (NPY) on basal and forskolin-stimulated cAMP levels in the rat cortex.

Results are expressed as means ± s.e.mean, n = 5.

* significant difference from control (basal) value

or as indicated by the bar

of cAMP were similar to the levels observed in the medulla oblongata, the magnitude of the elevation in cAMP accumulation in response to forskolin (10^{-5}M), was greater in the cortex. A significant reduction in the forskolin-stimulated cAMP levels was demonstrated in the presence of NPY (10^{-6}M) in the cortex, which is in agreement with the results obtained in the medulla (Figure 5.4).

5.3.3 Effect of Pertussis Toxin Pretreatment on the Neuropeptide Y-mediated Inhibition of cAMP Accumulation in the Medulla Oblongata

The influence of NPY on basal and forskolin-stimulated cAMP levels was re-examined following intracisternal PTX (2 μg or 5 μg) treatment and compared to the control situation where the animals were pretreated with saline (Figure 5.5). NPY (10^{-6}M) had no effect on basal levels of cAMP in both the vehicle and PTX (2 μg) treated animals. Although intracisternal pretreatment with a higher dose of PTX (5 μg) elevated basal levels of cAMP compared to vehicle treated animals, NPY (10^{-6}M) again had no effect on this basal value. In contrast to the reduction of forskolin-stimulated cAMP accumulation afforded by NPY in the control animals pretreated with saline, no reduction in forskolin-stimulated cAMP levels was evident after PTX (2 μg or 5 μg) pretreatment (Figure 5.5).

5.4 Discussion

Investigations have shown that NPY administered intracisternally or injected into discrete regions of the medulla

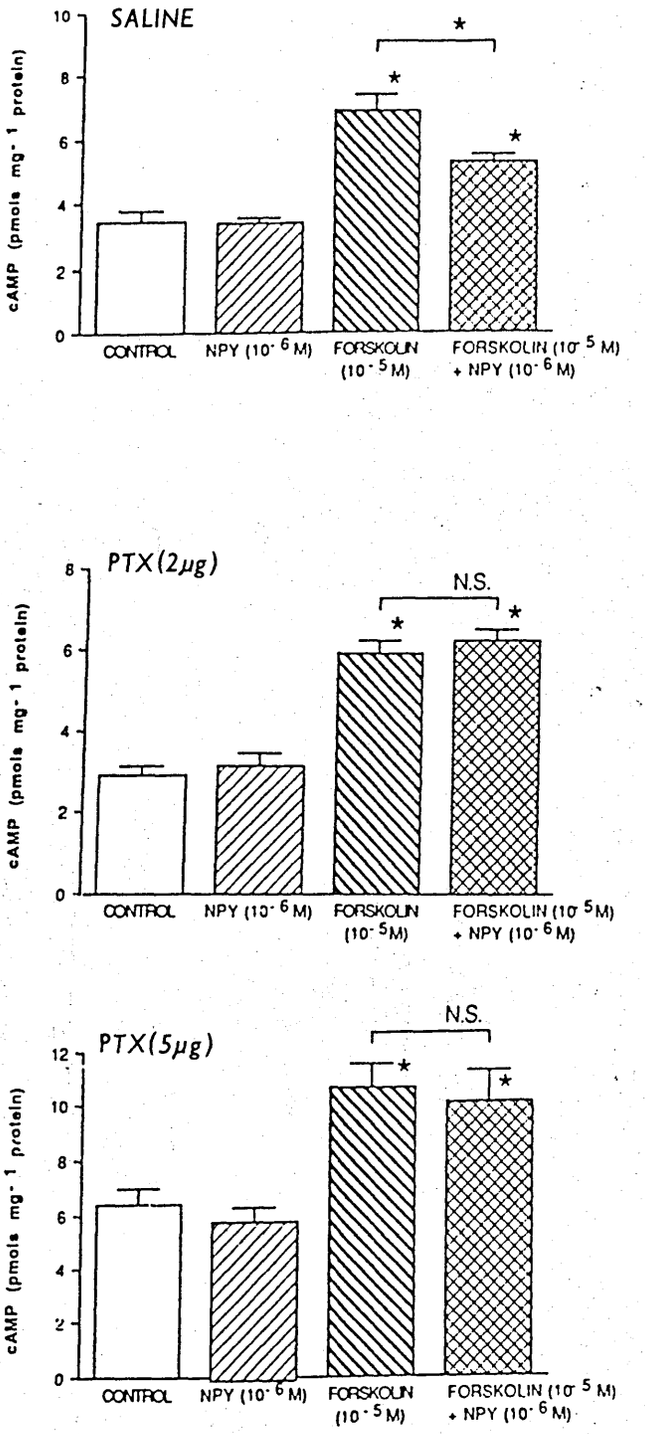


Figure 5.5

Effect of pertussis toxin (PTX) pretreatment on the neuropeptide Y-mediated inhibition of cAMP accumulation in the medulla oblongata.

Results are expressed as means ± s.e.mean, n = 7.

* significant difference from control (basal) value

or as indicated by the bar

oblongata in the rat produces a decrease in blood pressure and heart rate (Fuxe et al, 1983; Section 3.3). The present results suggest this effect may be mediated by a reduction in cAMP accumulation. This inhibitory action of NPY in the medulla oblongata was apparent when adenylate cyclase activity was stimulated by forskolin (10^{-5}M). Forskolin directly activates the catalytic subunit of this enzyme without involving the regulatory G-proteins and is therefore an invaluable tool for assessing the inhibitory action of agents on cAMP levels (Gehlert et al, 1985; Nelson et al, 1988; Seamon et al, 1981; Seamon and Daly, 1981).

In agreement with the present study, Petrenko et al (1987) observed in rat hippocampal membranes, that the inhibitory effect of NPY on adenylate cyclase activity was more evident when the enzyme was stimulated by forskolin. Furthermore, these authors demonstrated a maximal inhibition with 10^{-5}M NPY which corresponded to a 22% decrease in forskokin stimulated adenylate cyclase activity. Although a direct comparison cannot be made since the techniques and expression of results differ between studies, a maximal inhibition corresponding to a 30% (approximate) reduction in forskolin-stimulated cAMP levels was observed in the present study. Moreover NPY was not observed to completely attenuate either the stimulated levels of cAMP in the present study or the stimulated adenylate cyclase activity in the study by Petrenko and colleagues. This finding probably reflects the view that not all adenylate cyclase molecules accessible to

forskolin are also coupled to NPY receptors. This view is supported by the finding that adenylate cyclase molecules, localised by [^{3}H]-forskolin binding sites, quantitatively exceed typical transmitter receptor protein concentrations 10 to 100 fold, consistent with the role of both a messenger and an amplifier of receptor signals (Worley et al, 1986). The cognate peptide PYY and the classical transmitter acetylcholine in the rat striatum as well as adenosine (A_1 receptor) in the hippocampus have also have also been observed to reduce adenylate cyclase activity and indicate that like NPY these agents also appear to be negatively coupled to the adenylate cyclase enzyme (Westlind-Danielsson et al, 1988; Dunwiddie, 1985).

The absence of NPY-induced changes in cAMP accumulation under basal conditions is in accord with Harfstrand et al (1987c). Using slices from the NTS region of the medulla they also observed that the inhibitory effect of NPY on cAMP levels was apparent only in the presence of forskolin. Therefore it is presumed that at least in the medulla a regulatory mechanism exists in which the inhibitory effects of NPY appear only under conditions of elevated cAMP production. In contrast, Westlind-Danielsson et al (1987; 1988) demonstrated an NPY-attenuation of basal adenylate cyclase activity in membranes from rat cortex and striatum. This disparity between studies may either in differences in the region studied, or in the technique used. Therefore, the effect of NPY on cAMP levels in cortical slices was examined in the present study and compared to the investigation by Westlind-Danielsson et al (1987) who utilised

rat cortical membranes. The inhibition of basal cAMP levels by NPY, previously reported by Westlind-Danielsson et al (1987) in membrane preparations of the rat cortex, could not be demonstrated in a slice preparation from the same area under the present conditions. The discrepancy between the studies could derive from a variation in experimental conditions which may include the tissue preparation. Although the brain slice could be considered a more physiological preparation than membranes, perhaps diffusion barriers presented by the brain slice result in the bioavailability of NPY being lower compared to the membrane preparation. Consequently the 'responsiveness' of the slice preparation would be reduced making it more difficult to observe an NPY-evoked reduction of basal cAMP levels. However a more likely explanation for Westlind-Danielsson and colleagues to be able to demonstrate an inhibitory effect of NPY on basal cAMP levels could be due to the fact that these authors observed a higher basal level of cAMP at the outset. Thus detection of the inhibitory action of NPY on this parameter would be easier. The higher basal values in that study may be the result of a stimulating factor present under the basal conditions after using the membrane preparation technique or the result of a higher assay sensitivity. Although an analogous method to the present study was employed to measure cAMP levels (competitive protein binding assay), these authors did not utilise the Amersham kit for this purpose.

It is interesting to note that in the present study although the basal levels of cAMP in the medulla and cortex were similar, the response to forskolin was greater in the cortex compared to the medulla. This phenomenon was also observed by Daly et al (1982) in the same preparations. Since forskolin activates adenylate cyclase directly, one might expect this agent to increase the basal levels of cAMP to the same extent in different regions (Seamon and Daly, 1981). It has been reported by Bahner et al (1989) in the rat that although the basal levels of cAMP were similar in each brain region, the amount of adenylate cyclase and its basal activity varied from area to area. It is therefore possible that variation in the amount of adenylate cyclase accessible to forskolin may account for the differences observed between the medulla and cortex. Indeed, it has been observed that the density of tritiated-forskolin binding sites in the rat *cortex* is greater than in the rat *medulla* (McAuley, unpublished observations). This suggests that a greater amount of adenylate cyclase is present in the cortex and may account for the enhanced forskolin-stimulated levels of cAMP observed in this region.

As discussed previously an essential component of neurotransmitter regulation of adenylate cyclase involves G-proteins (Section 1.4). The involvement of an inhibitory G-protein, in the NPY reduction of forskolin-stimulated cAMP, was investigated using pertussis toxin. This toxin ADP-ribosylates the -subunit of the inhibitory G-protein. The inhibitory receptors are consequently uncoupled from the adenylate cyclase

complex, thereby preventing receptor-mediated inhibition of cAMP (Katada and Ui, 1982; Katada et al, 1986). A salient feature of PTX is the latency which exists between toxin administration and the induction of its biochemical effects. This lag period, which varies between tissues, has been proposed to be due to a slow penetration of the toxin into the cell membrane (Burns, 1988). PTX (2 μg; 5 μg) was therefore injected centrally 20 - 24 hours prior to the start of the study to accommodate for the delay in the onset of action. These parameters were similar to those employed in previous investigations concerned with the central effects of PTX (Nomura et al, 1987; Aghajanian and Wang, 1986).

In the absence of pertussis toxin NPY (10^{-6}M) as expected from previous data (Section 5.3.1), reduced the forskolin-stimulated cAMP accumulation. When the animals were pretreated with PTX, the ability of NPY to inhibit the elevated cAMP levels was abolished. This is consistent with the toxin inactivating the inhibitory G-protein by ADP ribosylation, thus preventing the transduction of inhibitory signals to adenylate cyclase. Motulsky and Michel (1988) demonstrated a comparable action of PTX on the NPY-inhibition of forskolin-stimulated adenylate cyclase activity in human erythroleukaemia cells.

In agreement with other workers, PTX (5 μg) also enhanced the basal levels of cAMP as well as rendering the tissue more sensitive to forskolin, suggesting that PTX alleviates the restraining action of the inhibitory G-protein on basal activity (Kassis et al, 1987; Katada et al, 1982). Using gel

electrophoresis and autoradiographical techniques, PTX has been shown to ADP-ribosylate a 41-KDa protein in membranes from rat atrial cells (Kassis et al, 1987). This protein corresponds to the α-subunit of one of the inhibitory G-proteins, the Gi-protein. This suggests that the NPY attenuation of cAMP formation observed in rat atrial membranes by Kassis et al (1987) may be mediated via the Gi-protein. However, it is not clear at present whether the same form of inhibitory G-protein mediates the reduction of adenylate cyclase in both the rat atrial membranes and in slices from the rat medulla oblongata.

NPY receptors in the central nervous system have been shown to be coupled to other effector molecules including phospholipases, protein kinases and calcium channel by means of G-proteins (Hinson et al, 1988; Perney and Miller, 1988). Thus the central haemodynamic actions of NPY may be regulated by more than one second messenger system. However, the coupling of the NPY receptors to effector molecules such as phospholipases (involved in inositol phosphate production, Section 1.4) has not been demonstrated to date in central regions associated with haemodynamic regulation. Perney and Miller (1989) have reported a small increase in the production of inositol trisphosphate and diacylgycerol as well as an increase in the mobilisation of calcium from intracellular stores in the sensory rat dorsal root ganglion (DRG) neurones following NPY. The stimulatory effect of NPY on inositol trisphosphate formation was attenuated by PTX indicating the involvement of a PTX substrate such as Go or one of the forms of Gi in the response. An involvement of inositol

phosphate production in the enhancing effects of NPY on noradrenaline-induced contractions in the periphery has previously been proposed (Section 1.5.6). Perney and Miller (1989) also reported that NPY can attenuate the depolarisation-induced influx of calcium ions and release of substance P from DRG cells. They also suggested that the NPY receptor is coupled to the voltage-dependent calcium channels via an inhibitory-like G-protein, Go.

The coupling of NPY receptors to two or more second messenger systems purports the involvement of more than one receptor subtype. Indeed as discussed in Section 3.4 the existence of more than one NPY receptor subtype has been proposed based on the differential efficacy of an NPY analogue (NPY_{13-36}) for the binding to central NPY receptors and for eliciting central hypotensive effects (Sheikh et al, 1989; Fuxe et al, 1987a). However, this issue remains controversial since several other reports have found only one NPY binding site in the various central regions (Chang et al, 1985; Saria et al, 1985; Goldstein et al, 1986). Hinson et al (1988), utilising rat cortical, striatal hippocampal miniprisms, have demonstrated an increase in inositol phosphate production in the presence of NPY. Wahlestedt (1987) proposed that at least in the cortical tissue this response was a result of Y_1 receptor activation since NPY_{13-36} was ineffective. However, a contradictory report was published by Perney and Miller (1989). These authors found that NPY_{13-36} was an effective agonist in stimulating inositol trisphosphate

production in DRG neurones, implying that the putative Y_2 was involved in phosphoinoside hydrolysis. It is not known whether the same G-protein mediates both the inhibition of adenylate cyclase and the stimulation of phosphate production or whether separate G-proteins regulate each effect. Perney and Miller (1989) have reported that the reduction in Ca^{2+} currents evoked by NPY in the DRG neurones was mediated via the G protein, Go, suggesting that NPY receptors are coupled to more than one G-protein. The clarification of receptor(s) and second messenger system(s) involved in the central and peripheral actions of NPY requires further work and awaits the development of an NPY antagonist.

In summary, this chapter describes one of the possible second messenger systems, coupled to NPY receptors in the medulla oblongata. It is proposed that the previously discussed hypotensive action of NPY may involve the reduction of cAMP mediated via an inhibitory G-protein. The exact nature of the G-protein and the type(s) of receptor involved in the biochemical and functional responses demonstrated remain to be elucidated.

CHAPTER SIX

POSSIBLE INTERACTIONS BETWEEN NEUROPEPTIDE Y RECEPTORS AND ADRENOCEPTORS ON cAMP LEVELS IN RAT MEDULLA OBLONGATA

Chapter Six

Possible Interactions between Neuropeptide Receptors and Adrenoceptors on cAMP Levels in Rat Medulla Oblongata

6.1 Introduction

The purported functional interactions between neuropeptide Y (NPY) and adrenoceptors located in the central nervous system have been postulated to exist at the level of the recognition site, and/or at the level of the coupling device (Fuxe et al, 1984; 1987b). Evidence for the former comes from radioligand binding data which describe a small increase in the number of NPY binding sites in cerebral cortical membranes after chronic intraperitoneal treatment of rats with the α_2-adrenoceptor agonist clonidine (Goldstein et al, 1986). Although these results are in contrast to the reduction in iodinated NPY binding sites in the medulla oblongata, observed using autoradiographical techniques, following the in vitro or intraventricular administration of clonidine in rats, the potential for receptor-receptor interactions is highlighted (Fuxe et al, 1986; Harfstrand et al, 1989). In addition, the modulation between NPY receptors and adrenoceptors may take place at post-receptor processes. Current evidence, including the work presented in the preceding chapter, indicates that NPY can inhibit the adenylate cyclase, via a guanine nucleotide binding protein (Section 5.3., Petrenko et al, 1987; Westlind-Daniellson et al, 1988). A similar mechanism of action has been proposed for peripheral α_2-adrenoceptors, which are also thought to be negatively coupled to

adenylate cyclase (Jakobs, 1985).

These findings, together with the demonstration of guanine nucleotide-induced changes in the equilibrium binding characteristics of both NPY and alpha-adrenoceptor agonists previously discussed in Section 5.1, suggest the possibility of an interaction between NPY receptors and α_2-adrenoceptors with regard to the inhibitory regulation of adenylate cyclase and cAMP production. Indeed, Harfstrand et al (1989) reported that in membrane preparations of the rat medulla oblongata, the reduction in the affinity of the α_2-adrenoceptor binding sites evoked by NPY was reduced in the presence of GTP. This is compatible with the view that NPY affects the binding of α_2-agonists via mechanisms that involve guanine-nucleotide binding proteins. Whether the modulation between these receptors can in turn regulate cAMP production has not been investigated to date in the central nervous system. However, it has been demonstrated in atrial and ventricular cardiomyocytes that NPY can inhibit isoprenaline-stimulated cAMP accumulation, suggesting a functional antagonism between neuropeptide Y and the β-adrenoceptor agonist isoprenaline in these tissues (Kassis et al, 1987; Cherie Miller et al, 1988).

In view of the obvious importance of gaining further insight into the understanding of the molecular mechanism of action and interaction of NPY and adrenoceptor agonists in the central nervous system, the current chapter is concerned with their possible interrelationship in the regulation cAMP in the medulla oblongata. Firstly, the actions of the α_2-adrenoceptor agonists

clonidine and moxonidine as well as the β-adrenoceptor agonist isoprenaline on cAMP accumulation were established. Secondly, the response to these agents in the presence of NPY was examined.

6.2 Methods

6.2.1 Preparation and Preincubation of Brain Slices from the Medulla Oblongata

The methodology used in the preliminary stages of the incubation experiments is as described in Section 2.8.1.

6.2.2 Drug Treatment of Slices - Alpha$_2$-Adrenergic Agonists

Following the initial incubation stage, the effect of clonidine on cAMP formation in slices of the medulla oblongata was investigated. The appropriate concentration of clonidine or vehicle was added to each tube in a volume of 20 µl and incubated for five minutes. This incubation was continued for a further ten minutes in the absence (basal values) or presence (forskolin-stimulated levels) of forskolin (10^{-5}M) as depicted in Figure 6.1. A congruous method was employed to examine the effects of moxonidine on basal and forskolin-stimulated cAMP formation. Furthermore, where the interaction between moxonidine (10^{-6}M) and NPY (10^{-6}M) was investigated, NPY was added simultaneously with moxonidine at the appropriate stage.

6.2.3 Drug Treatment of Slices - Beta-Adrenergic Agonists

At the end of the preliminary incubation stage, propranolol, NPY or appropriate vehicle was added and incubated for five

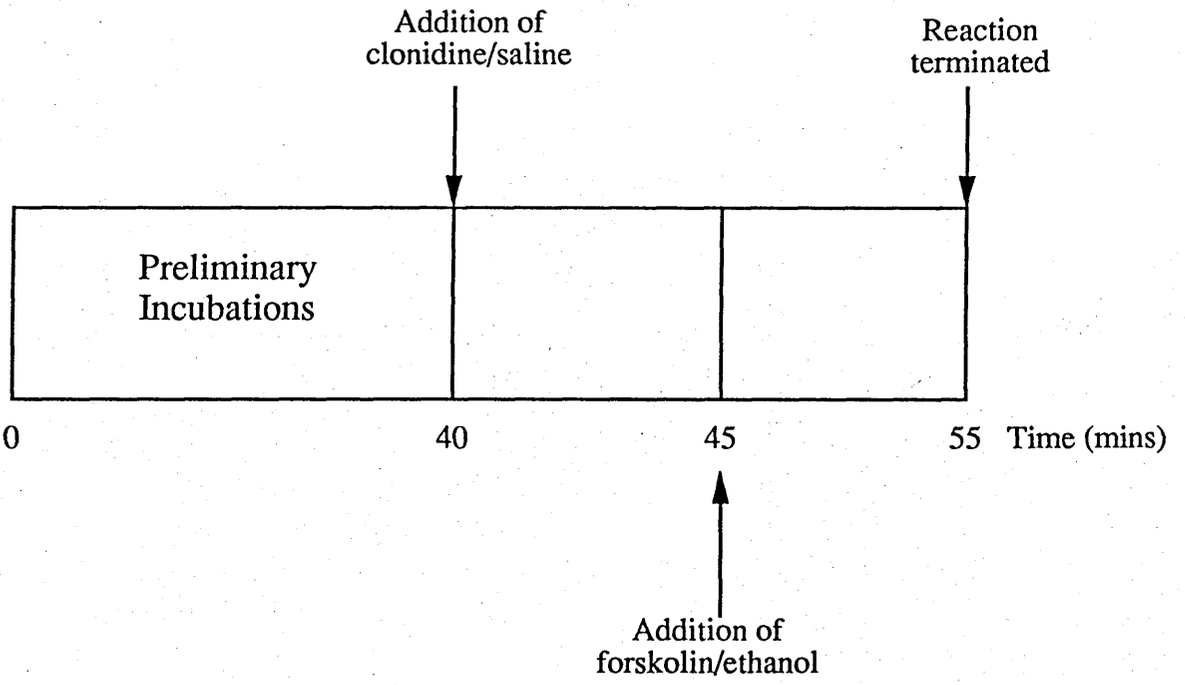


Figure 6.1

Protocol for the investigation of the effect of clonidine on basal or forskolin-stimulated cAMP levels in slices of the rat medulla oblongata.

minutes. Isoprenaline or vehicle (20 µl) was subsequently added and the incubation continued for a further ten minutes (Figure 6.2).

In all the experiments detailed above the reaction was terminated and cAMP levels measured as described in Sections 2.8.2 and 2.8.3.

6.2.4 Statistics

All values are expressed as means ± s.e.mean. The statistical test used to analyse this data was described previously in Section 5.2.3.

6.3 Results

6.3.1 Dose-Response Relationship of Clonidine on Basal and Forskolin-Stimulated cAMP Levels

The $alpha_2$-adrenoceptor agonist clonidine, over a range of concentrations from 10^{-8} - 10^{-5}M, did not significantly modify the basal cAMP levels in slices of the medulla oblongata (Figure 6.3). The effects of clonidine (10^{-7}M) were further examined on the elevated levels of cAMP produced by forskolin (Figure 6.4). Forskolin (10^{-5}M), as expected from previous data, significantly raised the levels of cAMP formation above control values. Clonidine (10^{-7}M), as well as having no effect on basal cAMP accumulation, did not alter the response to forskolin (Figure 6.4).

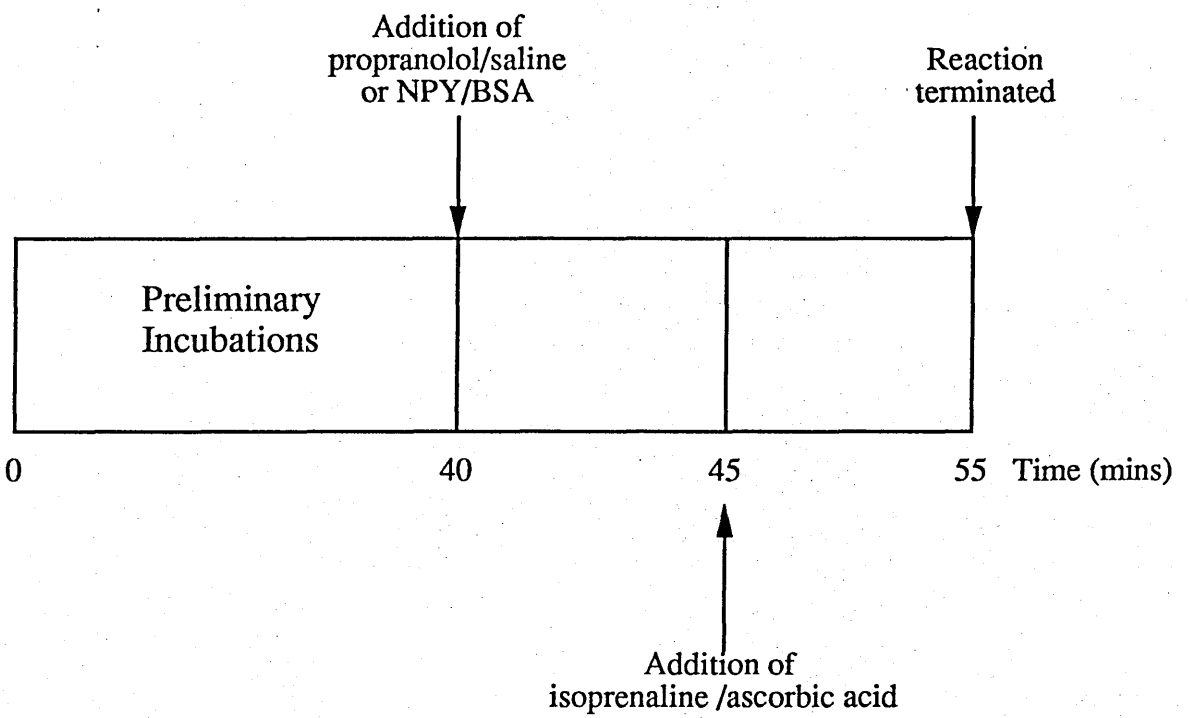


Figure 6.2

Protocol for the investigation of the effects of neuropeptide Y (NPY) or propranolol on basal or isoprenaline-stimulated cAMP levels in slices of the rat medulla oblongata.

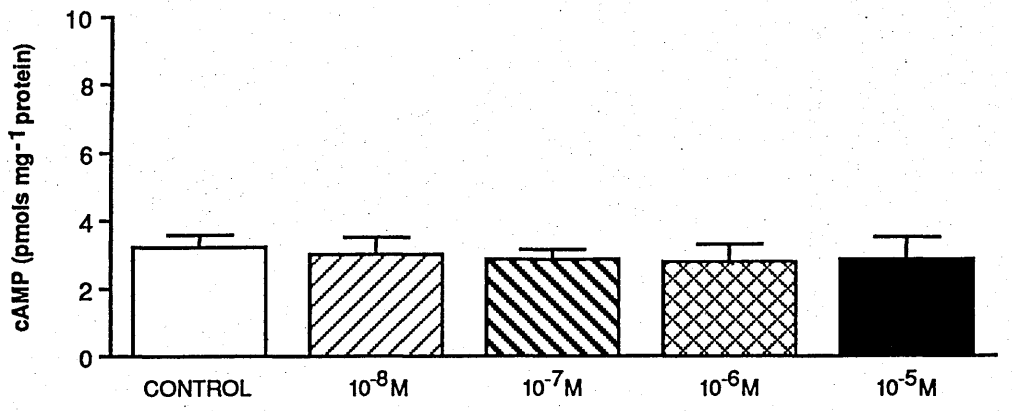


Figure 6.3

Lack of effect of clonidine (10^{-8} - 10^{-5}) on basal cAMP levels in the medulla oblongata.

Results are expressed as mean $\pm$ s.e.mean; n = 6.

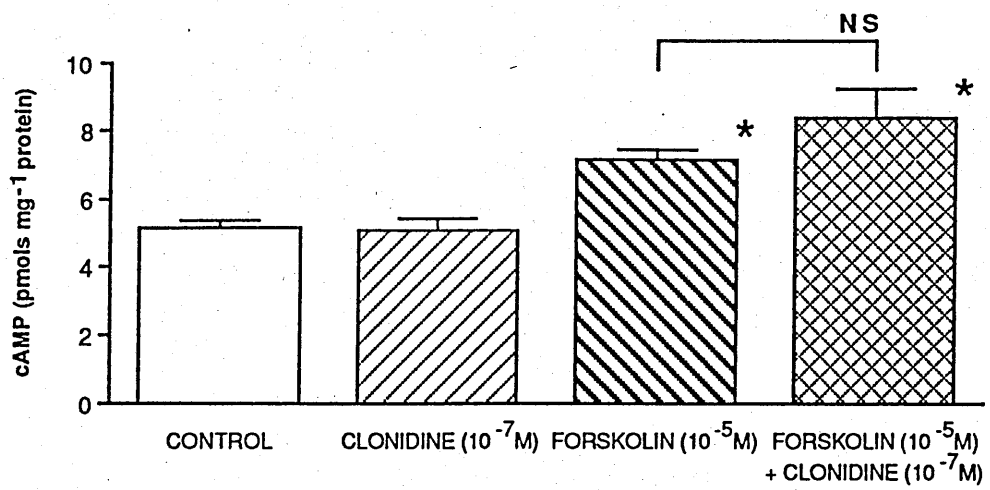


Figure 6.4

Lack of effect of clonidine (10^{-7}M) on basal and forskolin-stimulated cAMP levels in the medulla oblongata.

Results are expressed as mean $\pm$ s.e.mean; n = 6.

* significant difference from basal values ($p < 0.05$).

6.3.2 Dose-Response Relationship of Moxonidine on Basal and Forskolin-Stimulated cAMP Levels

As shown in Figure 6.5, moxonidine (10^{-8} - 10^{-5}M) had no significant effect on the basal levels of cAMP accumulation However, prior incubation with moxonidine (10^{-6}) significantly reduced the elevated levels of cAMP evoked by 10^{-5}M forskolin (Figure 6.6).

6.3.3 Possible Interaction Between the Effects of Moxonidine and NPY on Forskolin-Stimulated cAMP Levels

The incubation of moxonidine (10^{-6}M) in combination with NPY (10^{-6}M) had no effect on the basal levels of cAMP formation in slices of the medulla oblongata (Figure 6.7). Forskolin (10^{-5}M) again, significantly raised cAMP levels compared to basal values. Prior incubation of moxonidine (10^{-6}M) plus NPY (10^{-6}M) before the introduction of forskolin (10^{-5}M) did not significantly reduce the forskolin response (Figure 6.7).

6.3.4 Lack of Effect of NPY on Isoprenaline-Stimulated cAMP Formation

The β-adrenergic agonist isoprenaline (10^{-6}M) significantly increased the formation of cAMP in slices of the rat medulla oblongata as depicted in Figure 6.8. This effect of isoprenaline was significantly attenuated in the presence of propranolol (10^{-6}M) a specific β-adrenoceptor antagonist, which alone had no effect on basal cAMP accumulation (Figure 6.8). NPY (10^{-6}M) had no significant effect on basal levels of cAMP formation (Figure 6.9) and the incubation of NPY (10^{-6}M) prior to the

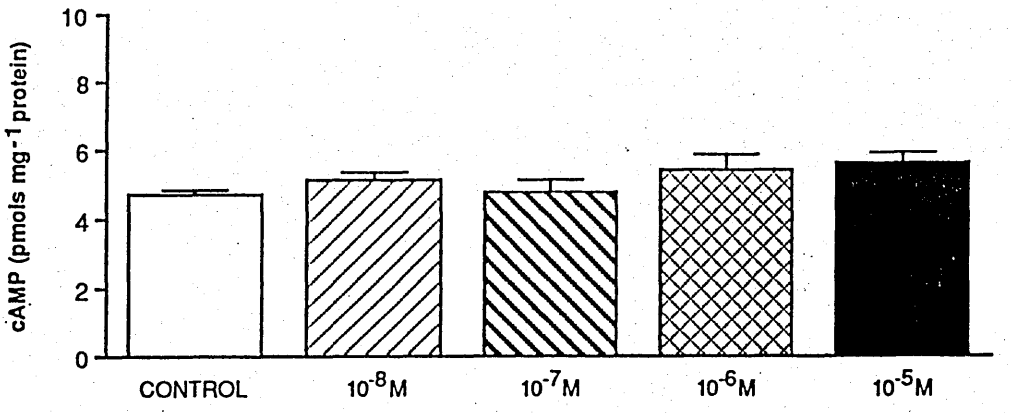


Figure 6.5

Lack of effect of moxonidine (10^{-8} - 10^{-5}M) on basal cAMP levels in the medulla oblongata.

Results are expressed as mean ± s.e.mean; n = 4.

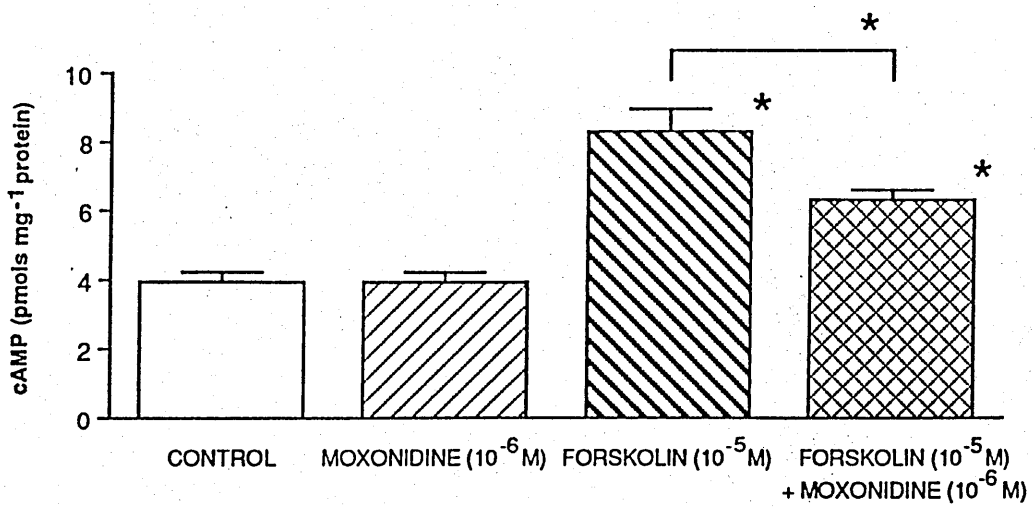


Figure 6.6

Effect of moxonidine (10^{-6}M) on basal and forskolin-stimulated cAMP levels in the medulla oblongata.

Results are expressed as mean $\pm$ s.e.mean; n = 6.

* significant difference from basal values or as indicated by the bar ($p < 0.05$).

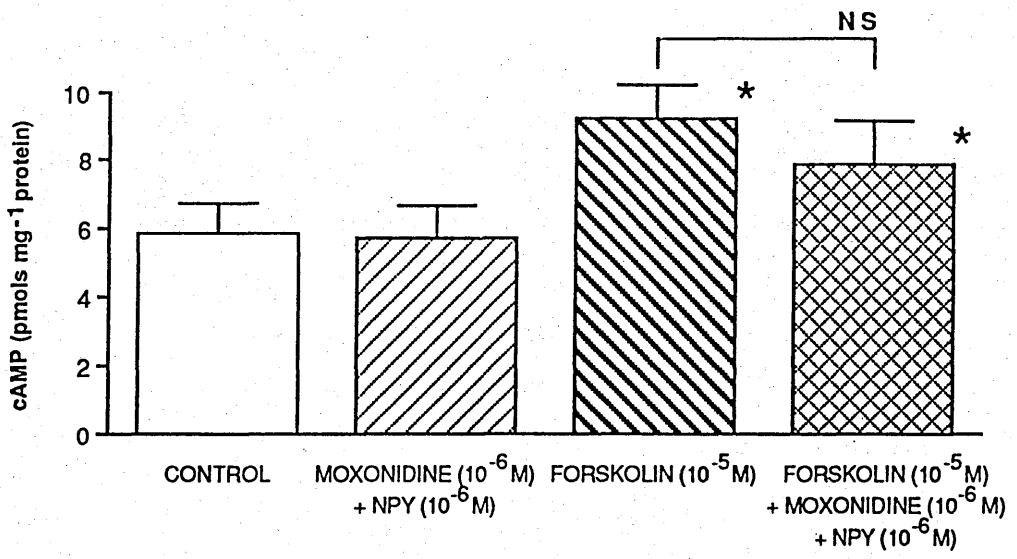


Figure 6.7

The effect of moxonidine (10^{-6}M) together with 10^{-6}M neuropeptide Y (NPY) on basal and forskolin-stimulated cAMP levels in the medulla oblongata.

Results are expressed as mean $\pm$ s.e.mean; n = 5.

* significant difference from basal value ($p < 0.05$).

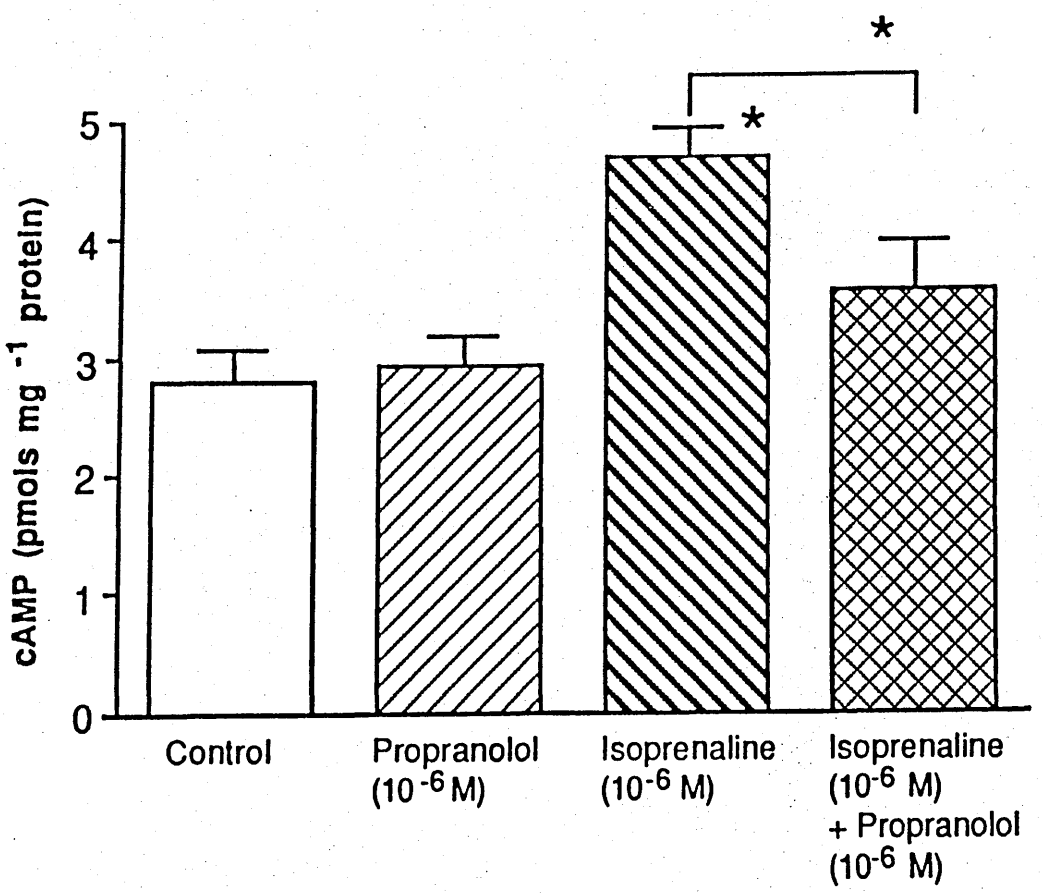


Figure 6.8

Effect of isoprenaline (10^{-6}M) in the absence and presence of propranolol (10^{-6}M) on cAMP levels in the medulla oblongata.

Results are expressed as mean $\pm$ s.e.mean; n = 9.

* significant difference from basal value or as indicated by the bar ($p < 0.05$).

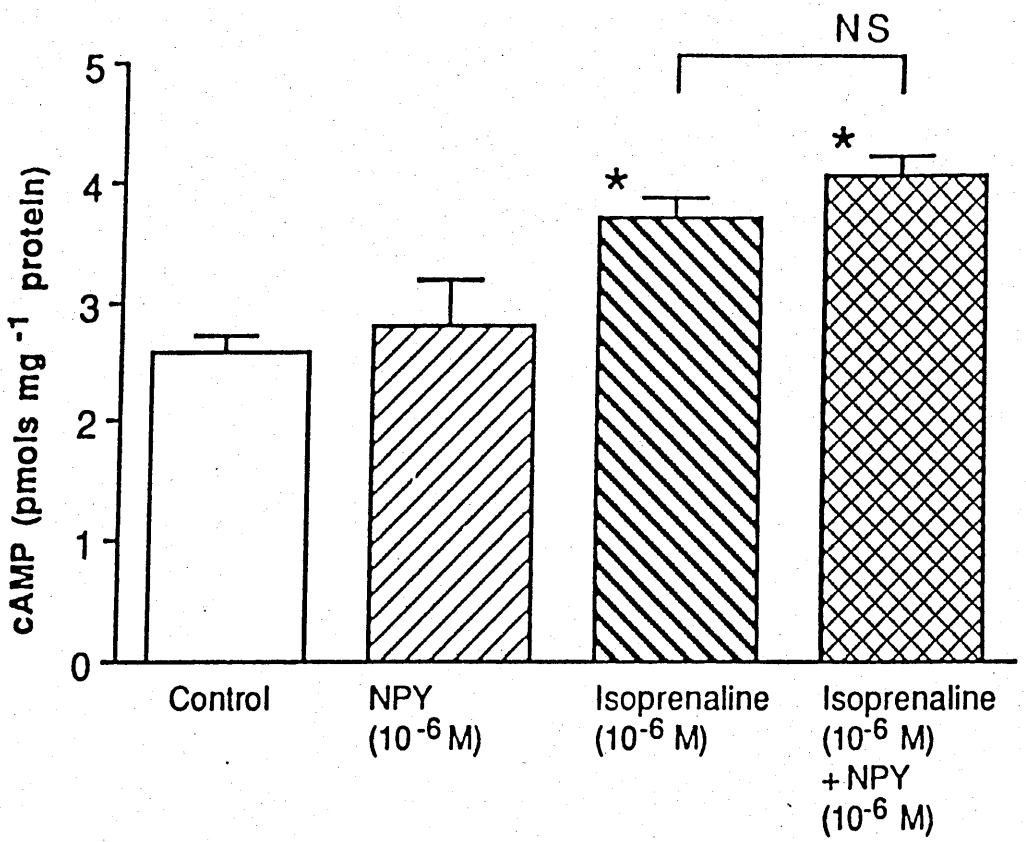


Figure 6.9

Lack of effect of NPY (10^{-6}M) on basal and isoprenaline-stimulated cAMP levels in the medulla oblongata.

Results are expressed as mean $\pm$ s.e.mean, n = 6.

* significant difference from basal value ($p < 0.05$).

addition of isoprenaline did not significantly modify the response to the latter agent (Figure 6.9).

6.4 Discussion

Although the negative coupling of α_2-adrenoceptors is well established in the peripheral tissues (Jakobs, 1985), the relation between cAMP and α-adrenoceptors in the central nervous system is more confusing. Initially it was reported that α-adrenergic agonists have little effect on cAMP accumulation in brain slices themselves but that they augment the response to substances that stimulate the enzyme (Daly et al, 1980; Pilc and Enna, 1985). This effect was later attributed to the stimulation of both α_1- and α_2-adrenoceptors (Pilc and Enna, 1986). More recently, Duman and Enna (1986) demonstrated that while the inhibitory effect of α_2-agonists on basal levels of cAMP was difficult to observe, it was possible to show the inhibitory response in the cerebral cortical slice when adenylate cyclase was stimulated directly with forskolin. The data obtained in the present study with the α_2-adrenoceptor agonist clonidine, indicates that in the medulla oblongata slice preparation this agent does not alter either the basal cAMP accumulation or the elevated levels of cAMP produced by forskolin. The inactivity of clonidine in this cAMP system may reflect its low intrinsic activity, since at least in the peripheral nervous system imidazolines such as clonidine (Figure 6.10) have been shown to be partial agonists at α_2-adrenergic receptors (Medgett et al, 1978; Armah, 1986). Indeed, a similar lack of effect of

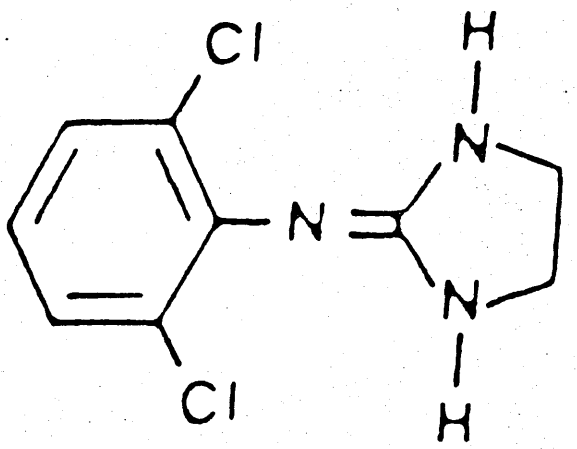


Figure 6.10

Structure of the alpha$_2$-adrenoceptor agonist clonidine

clonidine (10^{-5}M) was observed by Etgen and Petitti (1987) in the rat hypothalamic slice preparation. However, Duman and Enna (1986) observed that selective stimulation of α_2-adrenoceptors with clonidine, inhibited the forskolin-stimulated formation of cAMP in the cerebral cortex slice. In addition a similar inhibitory response to clonidine (10^{-5}M) which was reversed by the α_2-adrenoceptor antagonist yohimbine, was observed by Kitamura (1986) in particulate membrane fractions from the same area.

This implies that a regional variation in receptor populations may account for the inconsistency between investigations. Furthermore, intraperitoneal administration of clonidine in the rat has been observed to increase cAMP levels in the cerebellum, hypothalamus and medulla oblongata (Nakamichi et al, 1987). Due to the route of administration used by these authors the concentration of clonidine in the various brain regions cannot be determined and compared to the other studies. However, the fact that the α_1-adrenoceptor antagonist prazosin attenuates the response to clonidine in that particular study suggests that high concentrations of clonidine are present, which would reduce the selectivity for α_2-adrenoceptors and enable clonidine to act at α_1-adrenoceptors (U'Prichard et al, 1977; Timmermans et al, 1980).

Moxonidine, an imidazoline derivative (Figure 6.11), is a centrally acting antihypertensive agent, which, like clonidine, is considered to reduce blood pressure by an action on central

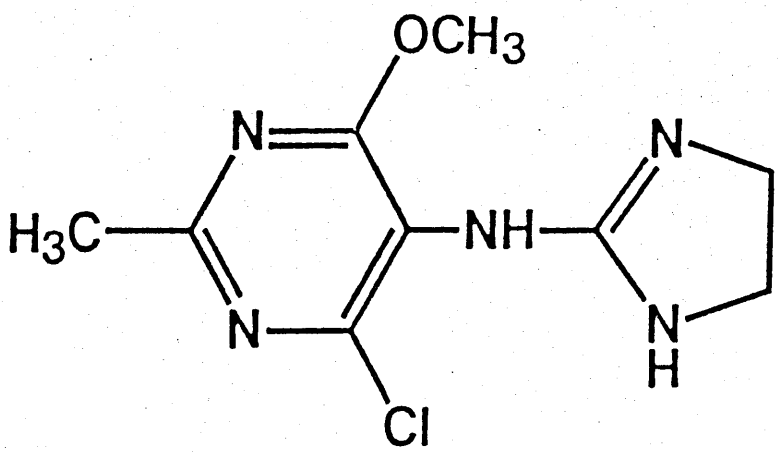


Figure 6.11

Structure of the alpha$_2$-adrenoceptor agonist moxonidine.

α-adrenoceptors (Armah and Stenzel, 1981; Planitz, 1987). Bergerhausen (1985), demonstrated that moxonidine was a full agonist at α_2 receptors on human platelet membranes with high potency and α_2-selectivity. Furthermore, in the isolated rabbit pulmonary artery moxonidine exerts a full α_2-agonist action, in contrast to clonidine which is shown to be a partial agonist in this model (Armah, 1986; 1987). Moxonidine in the present study, had no effect on the basal levels of cAMP but in contrast to the lack of effect observed with clonidine, moxonidine significantly reduced the elevated levels of cAMP produced by forskolin. A greater hypotensive effect has been observed following intracisternal administration of moxonidine compared to clonidine in anaesthetised rabbits (Armah, 1987).

The reason for the difference between the actions of clonidine and moxonidine on cAMP accumulation is curious since radioligand binding studies in cerebral cortex membranes have demonstrated the K_I value for displacement of (^{3}H) rauwolscine from α_2-binding sites was 3.48 x 10^{-7}M for moxonidine, compared to 2.06 x 10^{-8}M for clonidine, thus indicating that clonidine is the more potent agent (Armah, 1987). However, the discrepancy may be attributed to differences in the synaptic location of the site of action of these agonists. Indeed BDF 6143, an α_2-adrenoceptor antagonist with a higher presynaptic selectivity and potency than yohimbine, antagonised the hypotensive effects of intracisternally administered moxonidine in anaesthetised rabbit to a greater extent than the hypotension evoked by clonidine. Similarly, catecholamine depletion with 6-hydroxydopamine reduced

the hypotensive action of moxonidine but not clonidine in anaesthetised rabbits. This suggests that moxonidine elicits its hypotensive action via activation of presynaptic α_2-adrenoceptor sites (Armah, 1987), while clonidine may require post-junctional α_2-adrenoceptors to elicit its hypotensive effect. In guinea-pig spinal cord slices where the α_2-adrenergic receptors are proposed to be located post-synaptically, clonidine reduced forskolin-stimulated cAMP production (Wikberg and Hajos, 1987; Uhlen and Wikberg, 1988). However, a presynaptic action of clonidine may be a possibility if the α_2-adrenoceptors are located on non-noradreneric neurones (Haeusler, 1982; Section 3.4.2).

To evaluate the possible functional interaction between NPY receptors and α_2-adrenoceptors, NPY and moxonidine were added to the system in concert. This combination had no effect on basal cAMP levels. Moreover the significant reduction of forskolin-stimulated cAMP levels in the medullary slices produced individually by both the α_2-adrenoceptor agonist moxonidine and NPY, as demonstrated in Figures 6.6 and 5.3, was no longer apparent when these agents were incubated together (Figure 6.7). However, in the combination experiment the basal levels of cAMP and data variability were higher than in either of the individual studies, perhaps making it more difficult to observe a reduction in the forskolin-stimulated cAMP levels. Further studies to examine the correlation between the degree of forskolin-stimulated cAMP levels (above basal) and the magnitude of the

inhibition observed may be warranted. These biochemical data, although modest in degree, suggest an inhibitory interaction exists between NPY and moxonidine at the adenylate cyclase level and augment previous autoradiographical studies that purport a recipricol modulatory relationship between the respective receptors. Harfstrand et al (1989) reported that NPY (10 nM), in vitro or after intraventricular administration in consious rats significantly decreased the α_2-adrenergic binding sites (tritiated para-amino clonidine as a radioligand) in the NTS region of the medulla oblongata. Analagous studies in the same area revealed a decrease in the binding of iodinated NPY in the presence of 10 nM clonidine in vitro studies and in vivo studies subsequent to intraventricular administration (Fuxe et al, 1986; Harfstrand et al, 1989). Using radioligand binding techniques it was further demonstrated that in vitro the most consistent change in the binding characteristics of tritiated para-amino clonidine in the presence of NPY was a reduction in the affinity of the α_2 binding sites. This is in contrast to the previously reported increase in the number of α_2-adrenergic binding sites (Harfstrand et al, 1989; Agnati et al, 1983 Fuxe et al, 1986). It is presently unknown if the observed reduction of the (^{125}I)-NPY binding represents a reduction of the number or a reduction in the affinity of the (^{125}I)-NPY binding sites. The reduced affinity of the α_2-adrenergic receptor induced by NPY may reflect a diminished efficiency of this receptor. This view corroborates the present findings, since an antagonism of the individual inhibitory actions on forskolin-stimulated cAMP

accumulation was observed when NPY and moxonidine were added in concert, thus implying the existence of a receptor-receptor interaction between NPY and α_2-adrenoceptors.

While the existence of β-adrenoceptors in the rat central nervous system and their involvement in central cardiovascular regulation is known (Bylund and Snyder, 1976; Stone and U'Prichard, 1981; Borkowski and Finch, 1979; Peres-Polon and Correa, 1987), most studies investigating the central interaction of NPY and catecholamines have centered on the relationship between NPY and α_2-adrenoceptors. However, it has been demonstrated in ventricular myocytes isolated from the rat heart, that NPY (10^{-9} - 10^{-6}M) can reduce the magnitude of cAMP accumulation elicited by the β-agonist isoprenaline suggesting an interaction in this tissue between NPY receptors and β-adrenoceptors (Cherie Millar et al, 1988).

In slices of the rat medulla oblongata isoprenaline (10^{-6}M) increased cAMP accumulation through stimulation of β-adrenoceptors. The prospect of a functional interaction between NPY receptors and β-adrenoceptors was subsequently explored by adding NPY to the incubation prior to isoprenaline. The elevation in cAMP levels achieved by isoprenaline was not altered by NPY (10^{-6}M). It would therefore appear that at least in this system, NPY does not interact directly with β-adrenoceptor function. In agreement with the present study the binding characteristics of the radioligand dihydroalprenolol to β-adrenoceptors in membranes of the medulla oblongata were not

modified in the presence of NPY (Agnati et al, 1983). These results differ from the studies of a human neuroblastoma SK-N-MC cell line as well as cardiac atrial and ventricular cell lines, where NPY (10^{-6}M) significantly inhibited the response to isoprenaline suggesting an interaction between the respective receptors (Olasmaa et al, 1987; Kassis et al, 1987; Cherie Millar et al, 1988). Moreover, in the latter study it was shown that NPY was able to prevent both the accumulation of cAMP stimulated by isoprenaline in the ventricular cardiomyocyte and to reduce a pre-elevated cellular content of cAMP. However, the ability of NPY to inhibit isoprenaline-stimulated adenylate cyclase is not a ubiquitous occurrence since in addition to the present study the peptide had no effect on β-adrenergic stimulation of adenylate cyclase in membranes from certain cultured cells including rat glioma C 6 (Kassis et al, 1987). The variation between studies may be explained by the hypothesis that not all cell types possess both NPY and β-receptors that can interact. In this respect it has been demonstrated that in rat frontal cortex membrane preparations fused with Friend erythroleukemia cells, NPY was not able to inhibit the vasoactive intestinal polypeptide-induced elevation of cAMP production (Olasmaa and Terenius, 1986). Furthermore, these authors reported that in pilot experiments, NPY does not interfere with the function of PGE_1 receptors again suggesting that the juxtaposition of receptors does not always result in an interaction (Olasmaa and Terenius, 1986).

In summary, these studies demonstrate the α_2-adrenoceptor agonist moxonidine but not clonidine reduces forskolin-stimulated cAMP levels in slices of the rat medulla oblongata. Furthermore, a modest functional antagonism exists between NPY and the moxonidine in this system. However, an interaction between NPY and the β-adrenoceptor agonist, isoprenaline was not observed.

CHAPTER SEVEN

HAEMODYNAMIC ACTIONS OF ENDOTHELIN-1

Chapter Seven

Haemodynamic Actions of Endothelin-1

7.1 The Haemodynamic Effects of Intravenous Administration of Endothelin-1 in the Anaesthetised and Conscious Rat

7.1.1 Introduction

The present chapter is principally concerned with the possible central cardiovascular actions of the novel peptide endothelin-1 (ET-1). At the inception of this study a very limited amount of information was available pertaining to the haemodynamic effects of the peptide *in vivo*. In the inaugural report by Yanagisawa *et al* (1988a) which described the isolation and identification of ET-1, the authors also demonstrated that in the anaesthetised chemically denervated Sprague-Dawley rat, intravenous injection of ET-1 caused a transient decrease of systemic arterial pressure followed by a pronounced and sustained elevation in blood pressure. Although a biphasic cardiovascular response to systemic administration of ET-1 has since been reported by other groups in both the anaesthetised and conscious Wistar rat, the composition of this response to ET-1 appears to be influenced by such factors as the route of administration, anaesthetic state and basal blood pressure of the animal (de Nucci *et al*, 1988; Gardiner *et al*, 1989; Winquist *et al*, 1989). The aim of the current study was therefore to obtain preliminary data on the intravenous dose-response relationship to ET-1 in the anaesthetised and conscious Wistar-Kyoto rat. In the initial absence of data on the central actions of ET-1, this study would

provide a basis from which a reasoned dose range for the central administration of ET-1 could be estimated. Furthermore, the information obtained would enable comparison to be made between the peripheral and potential central cardiovascular actions of this peptide in the Wistar-Kyoto rat.

7.1.2 Methods

7.1.2.1 Intravenous Administration in Anaesthetised Animals

Experiments were carried out on male Wistar-Kyoto rats weighing between 270 - 285g. Anaesthesia was induced in a perspex box with halothane (5%) and maintained with (0.5 - 1%) halothane in a nitrous oxide/oxygen mixture (70 : 30%). A tracheostomy was performed and the animals' ventilation controlled by a small animals respirator. Polyethylene catheters, filled with heparinised saline (8 units ml^{-1}) were inserted into both femoral arteries, one for continuous blood pressure and heart rate measurement and the other for blood sampling. Using the same methodology a femoral vein was also catheterised for intravenous (i.v.) drug administration. Arterial blood samples were withdrawn for measurement of blood gases prior to drug administration. Ventillation was altered to maintain arterial carbon dioxide tension and arterial oxygen tension within normal limits. Body temperature was kept constant at $37^{o}C$ with an homeothermic blanket.

A stabilisation period of 30 minutes was then allowed before proceeding with the investigation. I.v. injections of ET-1

(1 nmol/rat) and saline (0.9% w/v) were given in a randomised order to each rat. All injections were delivered in a volume of 0.1 ml over a period of one minute.

7.1.2.2 Intravenous Administration in Conscious Animals

The conscious animal studies were performed on male Wistar-Kyoto rats weighting between 270 - 280g. The animals were prepared as detailed in Section 2.5 with arteries catheterised for arterial blood pressure recording and blood sampling, and vein for drug administration.

The animals were allowed to recover for three hours following surgery before commencing with the experiment. I.v. injections of ET-1 (0.1 - 1.0 nmol/rat) and saline (0.9% w/v) were administered in a randomised order, each animal receiving all the doses. The injection was given over a time period of one minute in a total volume of 0.1 ml.

7.1.2.3 Measurement of Plasma Catecholamines

Plasma catecholamine levels were determined (as described in Section 2.7) before and after (at the maximum blood pressure response) i.v. drug administration. This permitted an indirect measurement of sympathetic nerve activity.

7.1.2.4 Statistical Analysis

All results are expressed as mean $\pm$ standard error. Analysis of the data was carried out using Repeated Measures Analysis of Variance and where appropriate a Bonferroni correction was included for multiple comparisons.

7.1.3 Results

7.1.3.1 Effect of Intravenous Administration of Endothelin-1 on Blood Pressure and Heart Rate in the Anaesthetised Rat

Mean arterial pressure (MAP) and heart rate (HR) before ET-1 administration in the anaesthetised was 77 ± 4.4 mmHg and 237 ± 11.8 bpm, respectively. I.v. administration of ET-1 (1 nmol) produced a biphasic cardiovascular response as illustrated in Figure 7.1. An initial transient depressor response (64.0 ± 1.9 mmHg) occurred almost immediately but was not significantly different from the saline control value 30 seconds after the start of the injection. The phase was proceeded by a rapid and marked rise in blood pressure. The maximum response (142.4 ± 5.7 mmHg) was evident five minutes subsequent to i.v. ET-1 and the pressor effect was sustained for about two hours. The tachycardia associated with the initial hypotension varied in magnitude between animals, with the mean response (245.0 ± 9.2 mmHg) at 30 seconds post-injection not significantly different from the control values. The heart rate response returned to control values within 30 seconds and was not significantly different from the control value during the hypertensive stage of the blood pressure response. An example of a trace displaying the effect of i.v. ET-1 on arterial pressure and heart rate is shown in Figure 7.2.

7.1.3.2 Effects of Intravenous Administration of Endothelin-1 on Plasma Catecholamine Levels in the Anaesthetised Rat

Plasma noradrenaline and adrenaline levels following

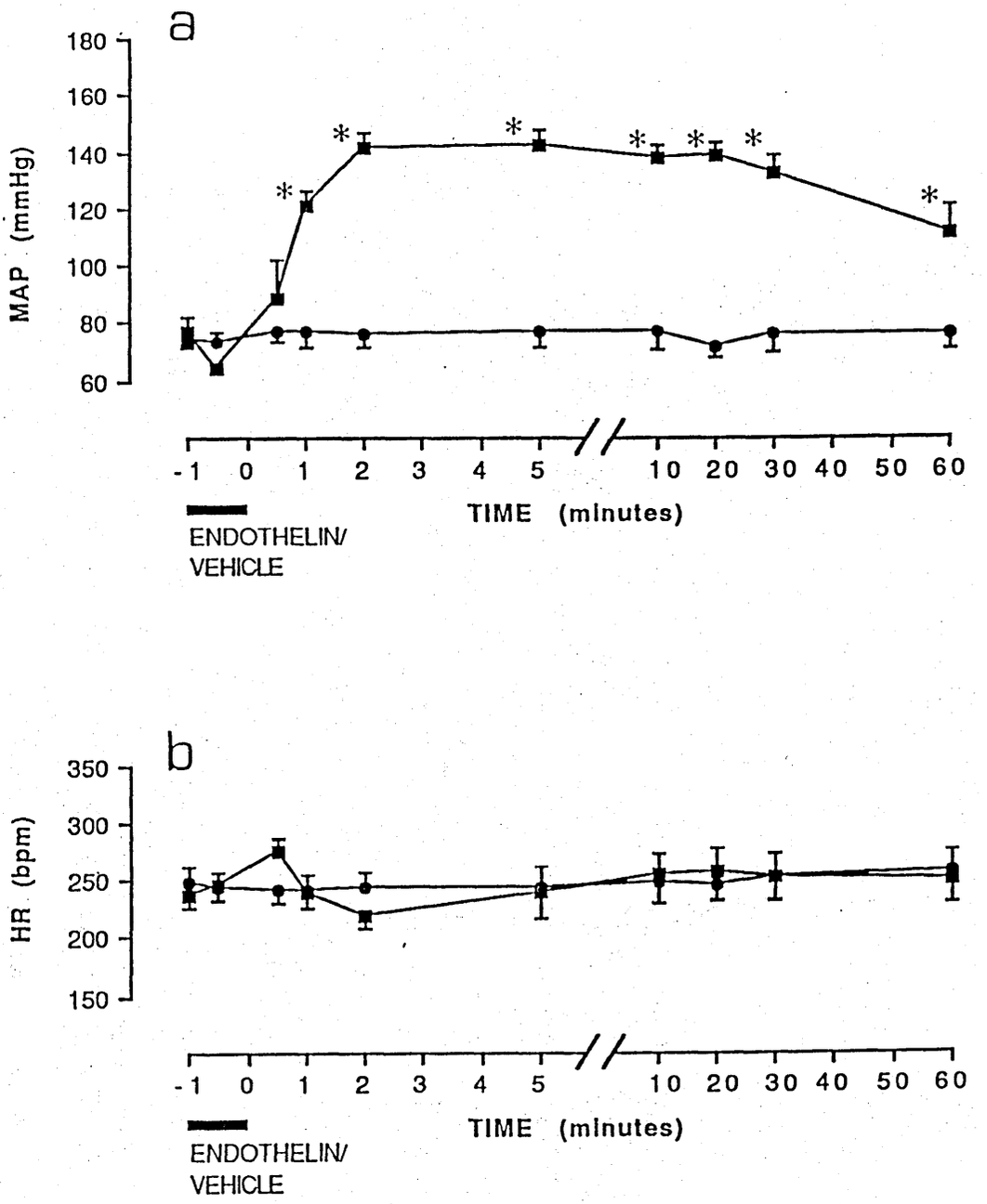


Figure 7.1

The effect of intravenous (i.v.) administration of ET-1 (1.0 nmol) on (a) blood pressure (MAP) and (b) heart rate (HR) of the anaesthetised rat.

Points represent mean data ± s.e.mean, n = 5

* significant difference from control value ($p < 0.05$)

● Saline

■ ET-1 (1.0 nmol).

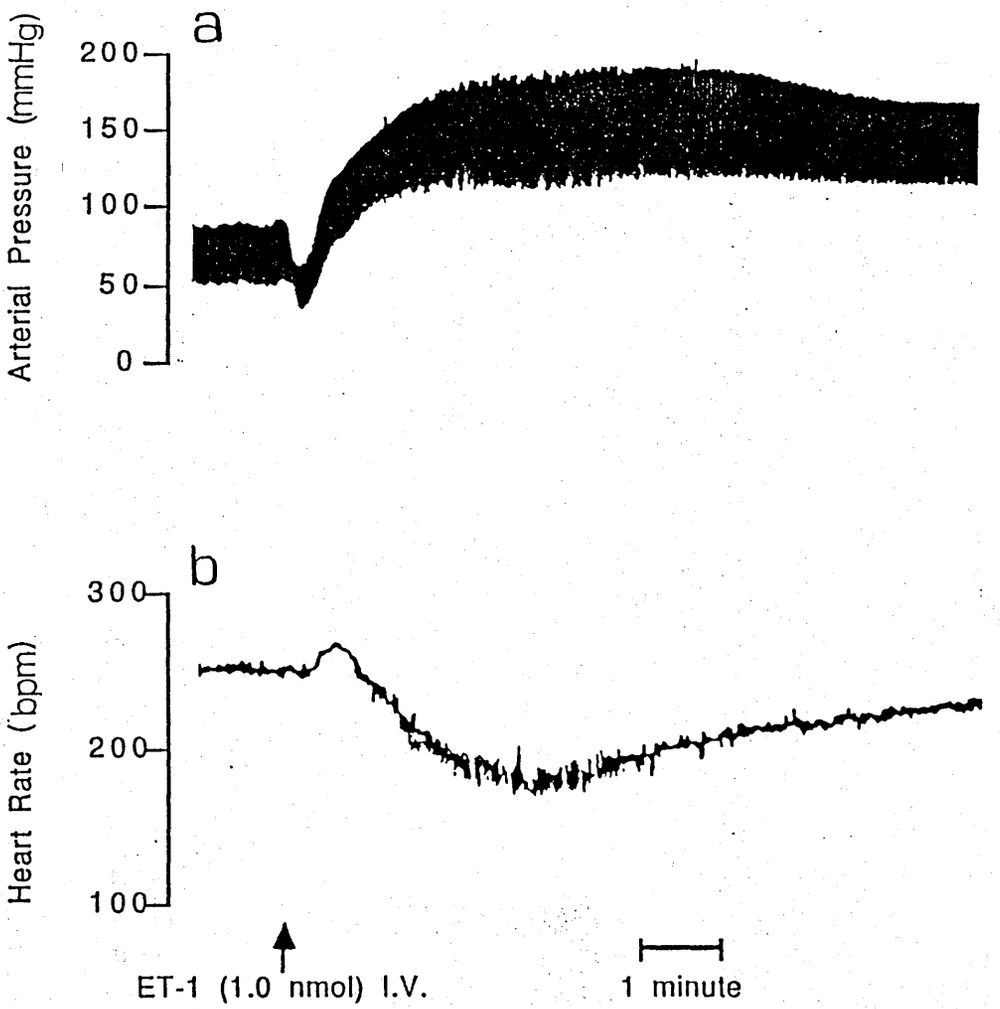


Figure 7.2

A representative trace of the (a) arterial blood pressure (mmHg) and (b) heart rate (bpm) following intravenous (i.v.) administration of ET-1 (1.0 nmol) in the anaesthetised rat.

1.0 nmol i.v. ET-1 were not significantly different from pre-injection values as demonstrated in Table 7.1. As expected, plasma catecholamine levels were not altered by i.v. saline injection (Table 7.1).

7.1.3.3 Effect of Intravenous Administration of Endothelin on Blood Pressure and Heart Rate in the Conscious Rat

The haemodynamic response to ET-1 was biphasic and dose-related over a range of 0.1 - 1 nmol (Figure 7.3). The MAP and HR were allowed to return to baseline values and stabilise between drug or vehicle injections. No significant difference was observed between the pre-injection values. The initial effect of the peptide was a transient dose-related fall in MAP. At the highest dose of ET-1 (1 nmol) this value (95 $\pm$ 5.1 mmHg) was significantly different from vehicle (127.8 $\pm$ 1.9 mmHg) 30 seconds after the start of the injection. The hypotension was followed by a gradual increase in MAP which reached a maximum between five and fifteen minutes depending on the dose and returned to control values within 60 minutes (Figure 7.3) The maximum blood pressure response to ET-1 (1 nmol), which occurred 15 minutes after administration was 151 $\pm$ 3.4 mmHg and was significantly different from vehicle (126.0 $\pm$ 4.0 mmHg).

A significant dose related elevation in HR was associated with the initial hypotensive phase (Figure 7.3). This response was greatest 30 seconds after i.v. ET-1 (0.3 - 1 nmol) and returned to control values within 2-5 minutes. At the highest dose of ET-1 investigated (1.0 nmol) the maximum HR observed was

Table 7.1

Lack of Effect of Intravenous Administration of Endothelin-1 (ET-1) on Plasma Catecholamine Levels in the Anaesthetised Rat

	Noradrenaline (nM)		Adrenaline (nM)	
	pre-injection values	post-injection values	pre-injection values	post-injection values
Saline	1.94 ± 0.91	1.68 ± 0.63	1.56 ± 0.84	1.71 ± 0.76
ET-1 (1.0 nmol)	1.61 ± 0.69	0.65 ± 0.19	2.97 ± 0.57	1.77 ± 0.56

Results are expressed as means ± s.e.mean

n = 5

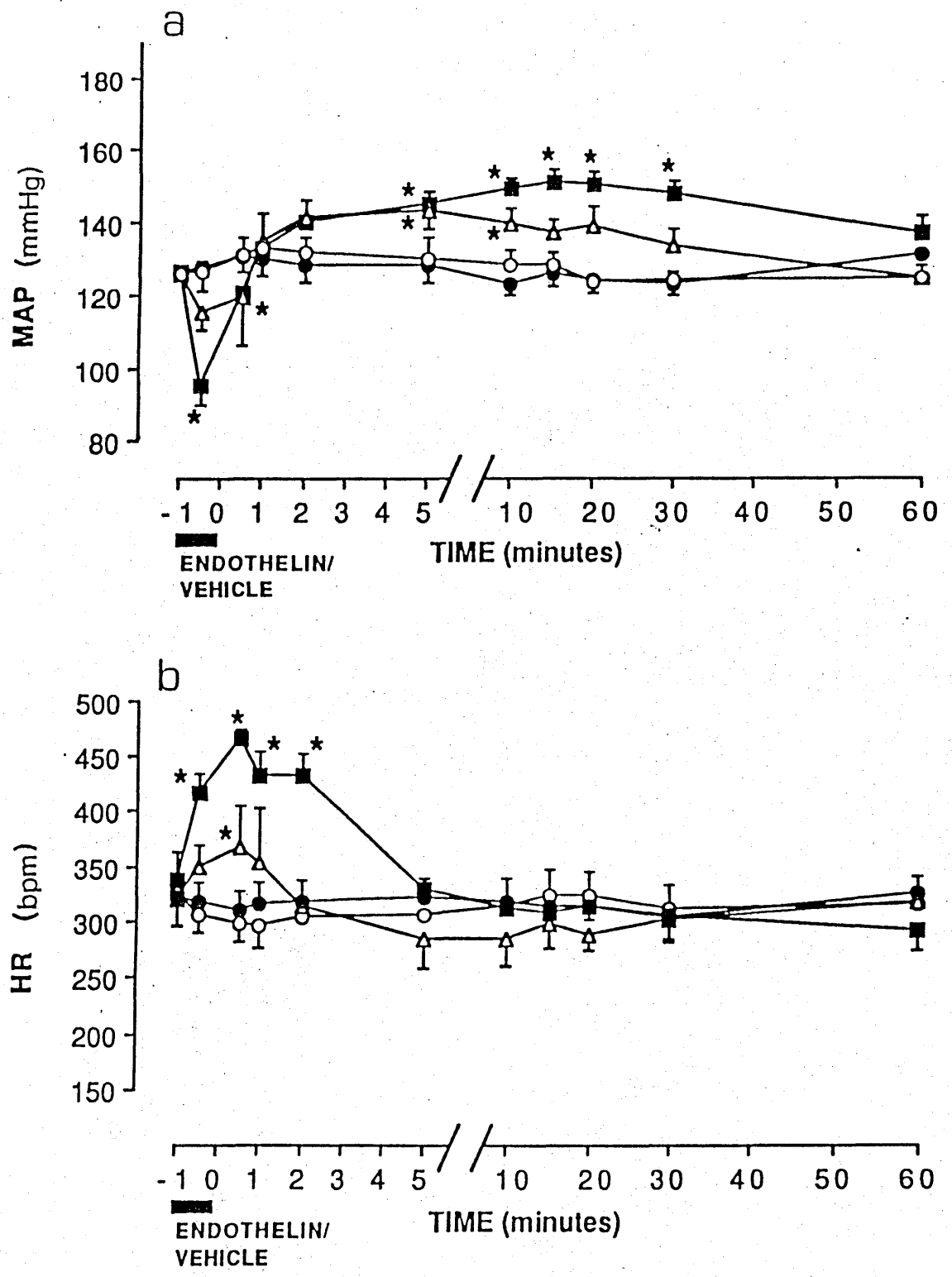


Figure 7.3

Effect of intravenous administration of ET-1 (0.1 - 1.0 nmol) on (a) blood pressure (MAP) and (b) heart rate (HR) in the conscious rat.

Points represent the mean blood pressure (mmHg) and heart rate (bpm) response ± s.e.mean, n = 5; * Significant differences from saline control value ($p < 0.05$).

● Saline
○ ET-1 (0.1 nmol)
△ ET-1 (0.3 nmol)
■ ET-1 (1.0 nmol)

467 ± 7.0 bpm compared to the control value of 309 ± 19.5 bpm. The haemodynamic effect of intravenous ET-1 in the conscious rat are illustrated in a representative response to 1 nmol ET-1 in Figure 7.4.

7.1.3.4 Effects of Intravenous Administration of ET-1 on Plasma Catecholamine Levels in the Conscious Rat

Plasma catecholamine levels were not significantly affected by i.v. injection of saline (Table 7.2) Similarly ET-1 had no effect on the levels of noradrenaline or adrenaline in the plasma (Table 7.2).

7.1.4 Discussion

A biphasic cardiovascular response to i.v. ET-1 was demonstrated in both the anaesthetised and conscious Wistar-Kyoto rat. These results corroborate those of Yanagisawa et al (1988a) in the chemically denervated, anaesthetised Sprague-Dawley rat. The mechanism underlying the initial depressor response has not been conclusively established. However, recent studies have reported that as well as eliciting a vasodilation in isolated blood vessels, ET-1 produces a transient regional increase in blood flow in vivo (Warner et al, 1989). The vascular beds dilated by this peptide include the hindquarters and carotid but the vascular reactivity and region affected appears to be dependent on the dose and route of administration (Gardiner et al, 1989; Han et al, 1989). In the present study, although ET-1 (1.0 nmol) elicited an initial decrease in blood pressure, a low dose of ET-1 (0.3 nmol) administered to the conscious rat

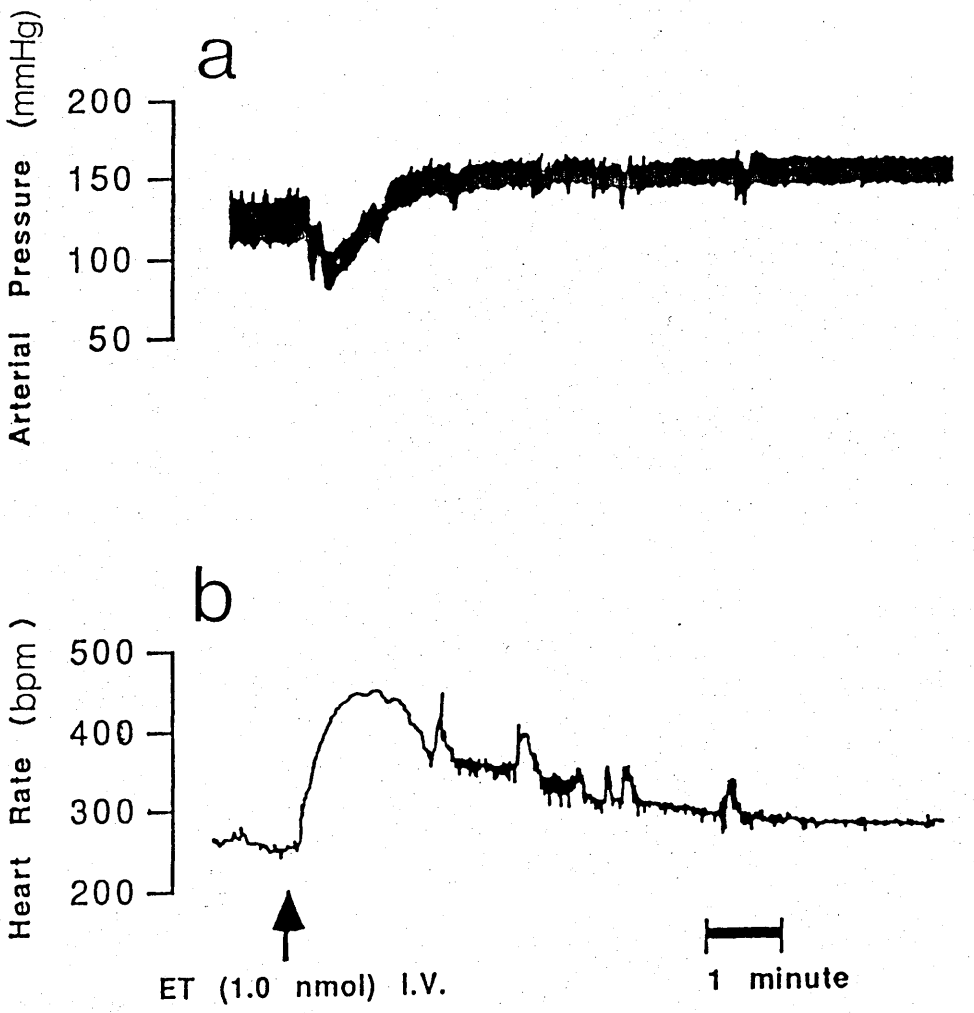


Figure 7.4

Representative trace showing (a) arterial blood pressure (mmHg) and (b) heart rate (bpm) following intravenous (i.v.) administration of ET-1 (1.0 nmol) in the conscious rat.

Table 7.2

Lack of Effect of Intravenous Administration of Endothelin-1 (ET-1) on Plasma Catecholamine Levels in the Conscious Rat

	Noradrenaline (nM)		Adrenaline (nM)	
	pre-injection values	post-injection values	pre-injection values	post-injection values
Saline	1.01 ± 0.25	0.91 ± 0.18	0.70 ± 0.29	0.78 ± 0.33
ET-1 (0.1 nmol)	0.87 ± 0.15	0.85 ± 0.21	0.72 ± 0.12	0.46 ± 0.19
ET-3 (0.3 nmol)	0.90 ± 0.12	0.60 ± 0.11	0.43 ± 0.08	0.71 ± 0.50
ET-1 (1.0 nmol)	0.79 ± 0.12	0.88 ± 0.19	0.93 ± 0.29	0.84 ± 0.22

evoked only a significant vasopressor response, indicating that at this dose vasoconstriction predominates.

The release of vasodilator substances by ET-1 has been proposed to account for the vasodilation observed in isolated tissues and may explain the depressor effect observed *in vivo*. In the rat perfused mesentery and lung preparations ET-1 has been shown to release endothelium-derived relaxing factor (EDRF) and prostacyclin (de Nucci *et al*, 1988). Moreover, the eicosanoid inhibitor indomethacin potentiates the pressor response to intravenous ET-1 in the pithed rat indicating the removal of a vasodepressor action of the peptide *in vivo* (de Nucci *et al*, 1988).

In the present study the observed tachycardia in the conscious rat was proportional to the initial fall in blood pressure suggesting it was reflex in origin and thus due to a reduction in vagal tone or increase in sympathetic nerve activity (Stornetta *et al*, 1987). Direct cardiac effects of ET-1 have also been reported including positive inotropic and chronotropic activity, ET-1 is also a potent secretagogue of atrial natriuretic peptide from rat atria (Ishikawa *et al*, 1988a; b; Hu *et al*, 1988). The latter action has also been postulated to contribute to the vasodepressor effect.

A difference was observed in the extent of the vasodepressor response elicited by intravenous ET-1 in the conscious rat compared to the anaesthetised rat. The smaller magnitude in the latter may be a consequence of the influence of anaesthesia on the factors discussed above and/or result from the prior

depression of vasomotor outflow by the anaesthetic, thus making it more difficult for the peptide to further reduce arterial pressure. Although the maximum pressor response to ET-1 (1 nmol) was similar in both groups, the magnitude of the rise in blood pressure was much smaller in the conscious animal. This may reflect activation of compensatory mechanisms in the conscious animal. Winquist et al (1989), have reported that the pressor response to i.v. ET-1 in the conscious animal was attenuated compared to the anaesthetised animal. These authors suggested that the vasoconstriction induced by ET-1 in the conscious animal may be offset by substantial decreases in cardiac output. De Nucci et al (1988), demonstrated that the pressor response subsequent to i.v. administration was more marked in the anaesthetised rat with low blood pressure (130/85 mmHg) compared those with high blood pressure (185/135 mmHg). These data further suggest that anaesthesia influences the vascular response to ET-1 in vivo.

A striking feature of the pressor phase of the in vivo response to ET is its time course. At least two hours were required for the return to pre-injection values after ET-1 (1 nmol i.v.) in the anaesthetised rat. In the conscious animals the hypertensive effect was sustained for a shorter period (one hour). The prolonged nature of the response is in agreement with other studies and has been shown to be associated with a time dependent vasoconstriction of a number of vascular beds including mesenteric, renal and hindquarter (Wright and Fozard, 1988; Han

et al, 1989). In the present study plasma catecholamine levels, an indirect determinant of sympathetic nerve activity, were not significantly altered in either the anaesthetised or conscious rat following i.v. ET-1. This would suggest that at least in the periphery this peptide acts directly on the vascular beds rather than interfering with sympathetic tone. Indeed, as discussed in Section 1.6, this direct effect on vascular smooth muscle cells may involve Ca^{2+}. However, the source of this Ca^{2+} remains controversial (Section 1.6.6). Specific receptors for ET-1 have been demonstrated on vascular smooth muscle cells (Hirata et al, 1988). The dissociation of the endothelin radioligand (^{125}I)-ET-1 from cultured rat aortic smooth muscle cells was very slow with 85% of the initial cell bound radioactivity still present after two hours (Hirata et al, 1988). Although internalisation of the peptide may contribute to this observation, the slow dissociation from the putative receptor may partly explain the long-lasting response in vivo.

Thus, in confirmation of the epoch study by Yanagisawa et al (1988a) in anaesthetised ganglion-blocked rats, ET-1 (1 nmol/rat) given intravenously elicits a biphasic cardiovascular response in both the conscious and anaesthetised Wistar-Kyoto rat. However, the composition of this response depends on the anaesthetic state of the animal. Moreover, in the absence of published data on the central activity of ET-1, an estimated range for the proposed studies on the central activity of the peptide (Sections 7.2 and 7.3) can be extrapolated from the doses utilised in the present study. In addition, a comparison of the peripheral and potential

central actions of ET-1 can be undertaken.

7.2 Haemodynamic Effects of Intracisternal Administration of Endothelin-1 in the Conscious Rat

7.2.1 Introduction

The first report of ET-1 constricting porcine coronary artery strips provoked a plethora of studies examining the peripheral acivity of this novel peptide (Yanagisawa et al, 1988a). Almost unnoticed in that initial study was a similar dose-response relationship to ET-1 in isolated cat basilar arteries, implying that endothelin may also regulate cerebral vascular function. Although the potent and prolonged vasoconstrictor activity of endothelin has since been extensively investigated in peripheral systems (Section 1.6.4), only a very limited number of reports have appeared concerning its effect on cerebral blood vessels.

Asano et al (1989) demonstrated in isolated canine basilar arteries, that ET-1 (10^{-11} - 3 x 10^{-8}M) induced a sustained dose-dependent vasoconstriction. The characteristic long-lasting nature of this response pointed to possible damage of the tissue by endothelin. However, the vasoconstriction was counteracted by the intracellular calcium antagonist, HA 1077, providing evidence that the vascular response was not due to irreversible damage (Asano et al, 1989). ET-1 binding sites have been localised very recently on cerebral vessels and it is possible that the observed slow dissociation of the endothelin from the putative receptor in

vascular smooth muscle cells may be instrumental in the prolonged response in cerebral as well as peripheral tissues (Hirata et al, 1988). An influx of calcium ions has been proposed as the mechanism for the slowly developing contractile response in the isolated middle cerebral arteries of the cat (Saito et al, 1989). In this tissue endothelin did not produce a vasoconstrictor response in a calcium-free solution. Moreover nicardipine and diltiazem, calcium channel blockers, attenuated the effect of endothelin by reducing the maximum responses but not the ED_{50} values implicating a non-competitive antagonism. Extracellular calcium ions are also required in certain peripheral blood, however, differences may exist in the mechanisms of channel opening between cerebral and peripheral blood vessels (Saito et al, 1989; Kurihara et al, 1989).

More recently, Koseki et al (1989a), using receptor autoradiographical techniques, observed that the distribution of binding sites for ET-1 subsequent to systemic administration of radiolabelled ET-1 were located only in the circumventricular structures of the median eminence and subfornical organ and choroid plexus located outside the blood-brain barrier. This is consistent with ET-1's inability to cross the blood brain barrier. A single class of specific high affinity binding sites ($Kd = 4\text{-}5 \times 10^{-10}M$) for ET-1 have however been demonstrated in membrane preparations from the rat brainstem (Koseki et al, 1989b). Moreover, these authors observed that in the brainstem, ET-1 binding was not displaced by various receptor agonists, toxins, peptides, and calcium channel blockers suggesting that

this peptide binds to a novel receptor.

As previously mentioned in Section 1.6.3, quantitative autoradiographical studies by Hoyer et al (1989) have revealed a differential pattern of ET-1 distribution in various brain areas studied. High densities of binding sites were observed in the hypothalamus and cerebellum with lower densities in the neocortex. The non-homogenous distribution within the rat brain regions indicate that the endothelin receptors may not only be located in blood vessels. The view that ET-1 binding has a neuronal rather than a vascular distribution is purported by Jones et al (1989). These authors have shown that regions of rat brain with a similar vascular supply display a differential pattern of distribution. Although there was no real evidence to support the assumption made by these authors, that ET-1 binding in the vascular tissue of each region is equivalent thereby any differences observed are due to parenchymal binding, the broad range of binding sites observed in central areas would suggest a more extensive role than control of vascular tone.

Thus, ET-1 could act in the central nervous system as a neurotransmitter or neuromodulator to control a variety of functions. The recent finding that ET-1 binding sites are present in the rat brainstem, an area of cardiovascular significance is worthy of particular attention since it suggests that this peptide may be involved in the central regulation of cardiovascular function (Koseki et al, 1989b). The present study investigated the cardiovascular actions of ET-1, by examining the

haemodynamic response to intracisternal administration of the peptide in the conscious rat. At the onset of this study no data was available concerning central administration of endothelin, therefore as a starting point, concentrations extrapolated from the intravenous dose-response relationship established in Section 7.1.3 were utilised in the proceeding experiments.

7.2.2 Methods

Conscious unrestrained male Wistar-Kyoto rats weighing between 275 - 300g were used in the experiments carried out in this section.

7.2.2.1 Preparation of Animals

Intracisternal guide cannulae were implanted prior to the study day as detailed in Section 2.6. On the day of the experiment, the femoral arteries were cannulated to monitor blood pressure throughout the procedure and to sample arterial blood (Section 2.5). Using the same methodology a catheter was also implanted in the femoral vein for i.v. administration of a fatal dose of pentobarbitone sodium (180 mg kg^{-1}), to kill the animal at the end of the procedure or it experienced any distress during the procedure.

7.2.2.2 Intracisternal Drug Administration

Intracisternal injections of vehicle saline (0.9% w/v) or 0.01 - 0.03 nmol/rat of ET-1, one injection per rat, were given via an injector, placed in the guide cannula, which was connected by tubing to a Hamilton syringe (5 μl). The injectate volume was

2.5 µl administered over 30 seconds. The lowest concentration (0.01 nmol) used in this dose range represents a 10-fold lower dose than the 0.1 nmol dose which was ineffective when given intravenously (Section 7.1.3). The narrow dose range in the current study reflects the difficulties experienced in finding endothelin concentrations producing cardiovascular effects without more profound centrally mediated effects.

7.2.2.3 Plasma Catecholamine Determination

Arterial blood samples were taken to measure plasma noradrenaline and adrenaline levels before and at the maximal blood pressure response to ET-1/vehicle administration (Section 2.7). This would allow an indirect determination of changes in sympathetic nerve activity following drug administration.

7.2.2.4 Statistical Analysis

The statistical test used to analyse the mean haemodynamic data was the Repeated Measures Analysis of Variance with a Bonferroni correction for multiple comparisons included where appropriate. Analysis of the changes in plasma catecholamine levels utilised the Kruskal-Wallis (non-parametric one way analysis of variance) statistical test.

7.2.3 Results

7.2.3.1 Effect of Intracisternal Administration of Endothelin-1 on Blood Pressure and Heart Rate

Intracisternal administration of ET-1 in conscious rats

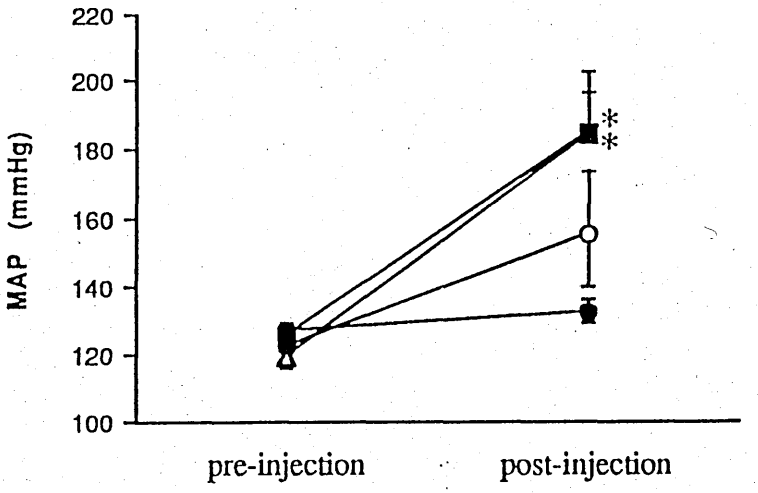
evoked complicated haemodynamic responses. Marked elevations in blood pressure in response to ET-1 (0.01 - 0.03 nmol) were observed in some but not all of the animal studies. Individual blood pressure and heart rate responses before and at the maximum (1-5 minutes) response after intracisternal injection are shown in Table 7.3. Data were not subdivided on the basis of blood pressure responses at this point. The mean data for each group is depicted in Figure 7.5. The pre-injection values of the control and treatment groups did not significantly differ from each other. As expected, intracisternal administration of saline did not significantly effect either blood pressure or heart rate. A prominent and significant rise in blood pressure was observed following administration of ET-1 (0.02 and 0.03 nmol) by the same route (Figure 7.5). In the animals where the increase in blood pressure to ET-1 exceeded 40 mmHg, this hypertension had a latency of onset between 0.5 - 1 minute. This hypertensive event was usually preceded by an increased activity of the animal which could develop into longitudinal rolling of the body (Table 7.3). If these events occurred or if the animal was in obvious discomfort, a fatal intravenous dose of anaesthetic was given. No consistent change in heart rate was demonstrated following intracisternal ET-1 (Figure 7.5). An example of a trace from an animal where administration of ET-1 (0.03 nmol) resulted in a dramatic rise in blood pressure is shown in Figure 7.6. In this animal the dose of ET-1 itself was lethal.

Table 7.3

Individual Blood Pressure Responses and Corresponding Heart Rate Values before and at the Maximum Response Following Intracisternal Injection of ET-1 (0.01 - 0.03 nmol) or Saline in the Conscious Rat

	Blood Pressure (mmHg)		Heart Rate (bpm)	
	Pre-injection	Post-injection	Pre-injection	Post-injection
Saline	131	135	305	310
n = 6	130	134	350	320
	130	140	315	370
	130	140	330	340
	115	120	410	410
	125	125	430	290
0.01 nmol	123	132	330	350
n = 5	115	125	270	270
	127	145	326	310
	125	165	300	270
	125	210*	320	-
0.02 nmol	124	135	300	330
n = 5	125	145	405	300
	118	220	315	390
	110	220	245	480
	118	200*	270	260
0.03 nmol	137	150	325	330
n = 7	120	145	300	300
	123	200*	345	290
	125	218*	320	270
	115	195	310	370
	125	215*	350	150
	130	180	300	330

* represents animals that experienced a concomitant barrel-rolling response



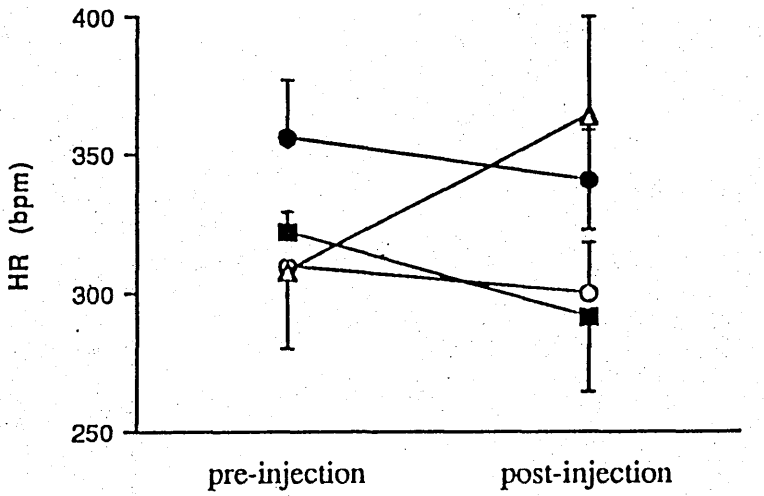


Figure 7.5

Effect of intracisternal administration 0.01 - 0.03 nmol endothelin-1 (ET-1) on blood pressure (MAP) and heart rate (HR) in the conscious rat.

Results are expressed as mean values ± s.e. mean, before and at the maximum response to intracisternal administration of saline or peptide; n = 5-7.

● Saline
○ ET-1 (0.01 nmol)
△ ET-1 (0.02 nmol)
■ ET-1 (0.03 nmol)

* Significant difference from saline control value ($p < 0.05$).

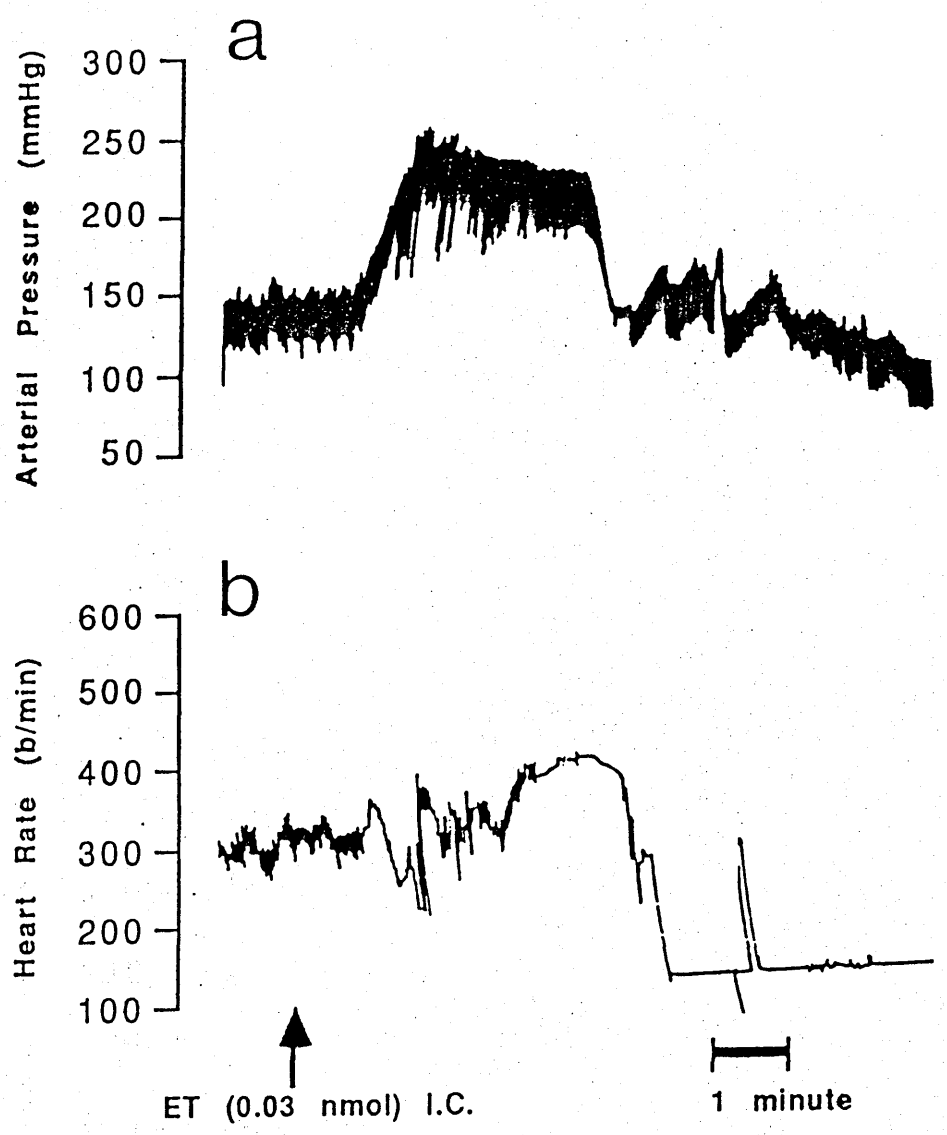


Figure 7.6

Representative trace of the (a) arterial blood pressure (mmHg) and (b) heart rate (bpm) following intracisternal (i.c.) administration of ET-1 (0.03 nmol) in the conscious rat.

7.2.3.2 Effects of Intracisternal Administration of Porcine Endothelin-1 on Plasma Catecholamine Levels in the Conscious Rat

Plasma catecholamine levels were determined before and at the maximum response to ET-1 or saline injection (Figure 7.7). An increase in plasma noradrenaline and adrenaline levels was evident following administration of the peptide and this reached significance with the highest dose of ET-1 (0.03 nmol). The variation in the data reflects the fact that catecholamine levels were increased only in those animals where the marked elevation in blood pressure was accompanied by marked changes in behaviour. The frequency of this response was previously shown in Table 7.3.

7.2.4 Discussion

Evidence has been presented in this section that ET-1 induces a pronounced hypertension following intracisternal (i.c.) administration in the conscious rat. The profile of this response differs from the previously demonstrated biphasic blood pressure response to i.v. injected ET-1, in both the conscious and the anaesthetised rat (Section 7.1.3). These data and the fact that 10-fold higher doses of ET-1 were required to elevate blood pressure when given by the i.v. route, imply that the hypertensive action of i.c. administered ET-1 is unlikely to be due to leakage of the peptide into the peripheral circulation. Since the completion of these studies a few reports have been published addressing the possible central activity of the peptide. Kawano et al (1989) demonstrated a similar difference

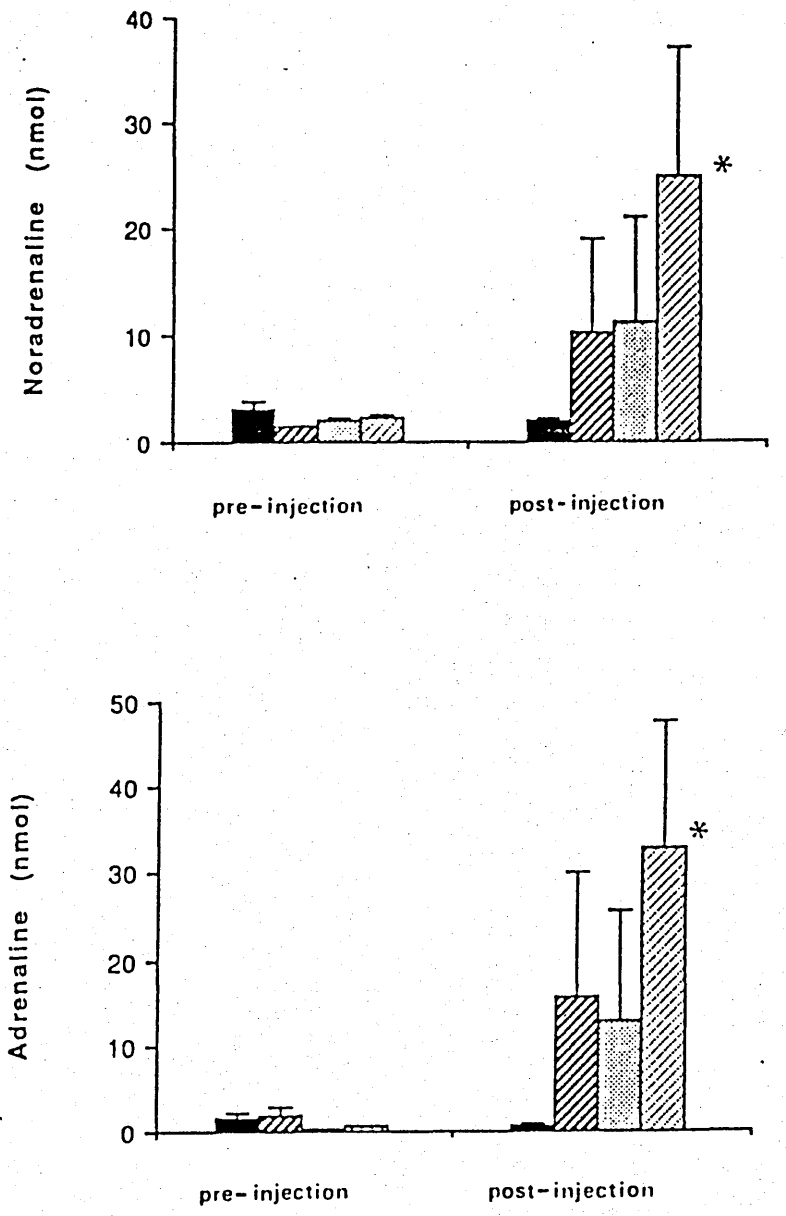


Figure 7.7

Effect of intracisternal administration of endothelin -1 (0.01 - 0.03 nmol) on plasma catecholamine levels in the conscious rat.

Data expressed as means ± s.e. mean, before and at the maximum response to intracisternal administration of saline or peptide; n = 5-7

■ Saline
▨ ET-1 (0.01 nmol)
▩ ET-1 (0.02 nmol)
▩ ET-1 (0.03 nmol)

* Significant difference from saline control value ($p < 0.05$)

between the doses needed to produce a rise in blood pressure via the intracerebroventricular (i.c.v.) compared to those needed via i.v. route, in conscious Wistar rats. These authors observed that i.c.v. administration of ET-1 at a dose of 100 ng/kg (0.04 nmol) produced an increase in blood pressure, whereas 1000 ng/kg (0.40 nmol) i.v. was needed to elicit a hypertensive effect. A tachycardia was observed following i.c.v. ET-1 in the latter study, however in the present investigations no clear-cut effect on heart rate was evident. The disparity between studies may be related to the dose of peptide used or the route of administration, i.c.v. concentrating the peptide's effects more rostrally.

Centrally mediated behavioural effects of ET-1 have also been demonstrated. Moser and Pelton (1989) have reported that ET-1 (0.005 nmol) injected into the right lateral ventricle produced a longitudinal rolling of the body analogous to that previously described for somatostatin (Cohn and Cohn, 1975). Cardiovascular parameters were not measured in the former study. However, it was reported that higher doses of ET-1, 0.01 and 0.02 nmol/rat, were lethal. In the current investigation intracisternal administration of the peptide (0.01 - 0.03 nmol/rat) elicited an increase in the blood pressure in some but not all of the animals. This response was variably accompanied by intense barrel-rolling movements and death. The consistent effects on motor coordination demonstrated by Moser and Pelton (1989) after i.c.v. injection, could be due to a greater concentration of the peptide around the cerebellum, the

area of motor coordination where a high density of endothelin binding sites have been reported (Jones et al, 1989). However, intracisternal administration of the peptide, although focusing the peptide's effects around the medulla, would still also concentrate the peptide's effects around the cerebellum. In contrast to the results of Moser and Pelton (1989) no overt behavioural activity was reported by Kawano et al (1989) following injection of the peptide by the same route. The lower concentration of ET-1 utilised in the latter study may explain the discrepant results.

The wide variation in the haemodynamic responsiveness to i.c. ET-1 is curious. However, a similar phenomenon has been reported by Wiklund et al (1988) in the femoral arteries from the guinea-pig. These authors observed only nine of 21 preparations contracted to endothelin (10^{-9} - 10^{-8}M) and again there was no obvious explanation. In the present study problems associated with the activity of the peptide can be excluded since the same batches of endothelin were used in the intravenous experiments carried out at the same time (Section 7.1.3). Although the patency of the cannula was confirmed before injection of the peptide (as described in Section 2.6) and the position of the cannula examined at the end of the procedure, it is possible that in some cases the delivery of the peptide from the cannula into the cerebral spinal fluid was impaired. This would account for the 'all or none' phenomenon observed. However as will be discussed in Section 7.3.4 further experiments are required to

verify this view.

An increase in sympathetic tone has been associated with the increase in blood pressure following injection of ET-1 into the ventricles of the conscious rat. Kawano et al (1989) demonstrated that pretreatment with intravenous hexamethonium (a ganglion blocker) significantly attenuated the rise in blood pressure produced by i.c.v. administration of ET-1. In the present study, plasma catecholamine levels were determined as an indirect measurement of sympathetic nerve activity. At the highest dose of ET-1 studied, a significant increase of both adrenaline and noradrenaline levels compared to control values, was observed. This would suggest that an increased sympathetic nerve activity mediated via central cardiovascular regulatory pathways was responsible for the hypertensive response evoked by the peptide. However, further examination of the data revealed that catecholamine levels were only elevated in the animals that experienced intense activity (longitudinal rolling) together with the hypertensive response. Therefore the interpretation of the catecholamine results is not straightforward and requires further and perhaps more direct investigation.

Autoradiographical studies in the brainstem have revealed ET-1 binding sites in areas corresponding to nuclei associated with cardiovascular regulation and body fluid homeostasis (Koseki et al, 1989a). These regions include the nucleus of the solitary tract, the rostral ventrolateral medulla, the parabrachial nucleus as well as the paraventricular and supraoptic nuclei of the hypothalamus. Kawano et al (1989) also observed that

intravenous administration of a vasopressin antagonist, attenuated the hypertensive response to the i.c.v. administration of ET-1, suggesting the involvement of vasopressin in the central effects of the peptide. Although the distribution of ET-1 binding sites in the brain was distinct from those of vasopressin, an additional link between these peptides has been proposed since the circumventricular structures bound by the systemic administration of ET-1 also contain binding sites for atrial natriuretic peptide. This latter peptide has been shown to be involved in the regulation of vasopressin secretion and its release is stimulated by ET-1 in guinea pig atria (Koseki et al, 1989b; Fukuda et al, 1988). Further evidence for a neuropeptide role for ET-1 in the central nervous system is suggested by the finding that ET-1-like immunoreactivity is present in certain types of neurones such as motor neurones and primary sensory neurones in the porcine spinal cord (Yoshizawa et al, 1989a). Moreover ET-1 has been shown to depolarise a ventral root potential in the rat spinal cord giving further support to a neuronal function for this peptide (Yoshizawa et al, 1989b). The distinctive hypertensive and behavioural responses seen in the present study could also be mediated via the previously described binding sites on cerebral blood vessels. Shigeno et al (1989) have demonstrated that intracisternal administration of ET-1 (5-500 pmol) resulted in a potent vasoconstriction of the feline and canine basilar artery as measured by angiographic techniques. Thus the present data can only be regarded as a starting point in

the investigation of the central haemodynamic actions of ET-1. Further detailed experiments are necessary to ascertain the exact mechanisms(s) involved in this putative role of ET-1. This issue is considered in the following section.

7.3 Effect of Intracisternal Administration of Endothelin-1 on Cerebral Blood Flow in the Anaesthetised and Conscious Rat

7.3.1 Introduction

The results of the preceding section (Section 7.2) demonstrate that intracisternal administration of ET-1 in the conscious rat evokes a marked elevation in blood pressure, with no consonant change in heart rate. This marked increase in blood pressure which accompanied the central administration of endothelin, may be secondary to an action on brainstem nuclei associated with cardiovascular control or a consequence of cerebral vasoconstriction. Indirect autoradiographic evidence supporting the former postulate has emerged very recently and has been discussed previously in Section 7.2.4.

An alternative mechanism for the distinctive hypertensive event might be a causal relation to the constriction of cerebral blood vessels. Asano et al (1989) demonstrated, using angiographic techniques, that intracisternal administration of ET-1 (10 - 1000 pmol) in anaesthetised dogs produced a sustained basilar artery vasoconstriction with an increase in blood pressure observed at the highest concentration. These in vivo findings, combined with in vitro studies on isolated cerebral

vessels, which demonstrate a prominent vasoconstrictor activity of ET-1 (Section 7.2), suggest that the dramatic changes in blood pressure and motor coordination observed following intracisternal injection of endothelin in rats (Section 7.2) may be a consequence of the reported potent and prolonged cerebral vasoconstrictor activity of ET-1.

The present study addressed this hypothesis by determining the regional cerebral blood flow following intracisternal administration of ET-1 in the rat.

7.3.2 Measurement of Cerebral Blood Flow in Anaesthetised and Conscious Rats - Methodology

Male Wistar-Koyoto rats weighing between 270 - 310 g on the study day, were utilised in the proceeding investigations.

7.3.2.1 Preparation of Animals

Anaesthesia was induced in a perspex box with halothane (5%) in a nitrous oxide/oxygen mixture (70% : 30%) and maintained with 0.5 - 1% halothane. A tracheostomy was performed when cerebral blood flow (CBF) was to be measured in anaesthetised animals. The animal's ventilation was controlled by a small animal respirator.

Polyethylene cannulae containing heparinised saline (8 units ml^{-1}) were inserted into both femoral arteries to monitor arterial blood pressure and sample arterial blood. Both femoral veins were also catheterised for administration of the radioactive tracer and where necessary, intravenous

administration of a fatal dose of anaesthetic. Arterial blood samples were withdrawn for measurement of blood gases prior to tracer administration. In anaesthetised animals, ventilation was adjusted to maintain arterial carbon dioxide tension ($PaCO_2$) between 32 and 40 mmHg. Animals with an arterial oxygen tension (PaO_2) of below 80 mmHg were excluded from the study. Rectal temperature was maintained at 37°C with an automated heating box.

When measurement of CBF was to be carried out on conscious animals the rats were anaesthetised and femoral cannulae implanted as above. The wounds were then infiltrated with lignocaine, a local anaesthetic and sutured. The animals were restrained in a plaster cast which extended from the front to the hind legs. The latter were further restricted from movement and the possible displacement of the cannulae, by taping to a fixed platform on which the animal was placed. The rats were then allowed to recover from the anaesthesia for a period of three hours before commencing with the experiment. Arterial blood samples were withdrawn as described above with the same blood gas criteria needed for entry into the study.

7.3.2.2 Intracisternal Drug Administration

Intracisternal injections of saline (0.9% w/v) or endothelin (30 pmols/rat) were made via an injector inserted into the implanted guide cannula, as described in Section 2.6. The dose of endothelin chosen for intracisternal injection, represented a concentration which evoked the most consistent hypertensive response in the previously described study on conscious rats

(Section 7.2.3). In accordance with the methodology used in Section 7.2.3, each rat received one injection of either the peptide or saline in a volume of 2.5 µl over a time period of 30 seconds.

7.3.2.3 Cerebral Blood Flow Measurements with the [^{14}C] - Iodoantipyrine Autoradiographic Technique

The effects of E-1 on cerebral blood flow (CBF) were first determined in anaesthetised rats. Since the timing of this procedure is critical and the end point timed exactly by decapitation using a guillotine, anaesthesia prevents any possible delays in the kill time due to movement of the animal. However, the results obtained from the first series of experiments on anaesthetised animals necessitated further examination of the effects of endothelin on cerebral blood flow in conscious animals. Since some of the animals were likely to experience behavioural effects which affected movement as described earlier, the procedure was terminated and the animal killed by bolus injection of euthetal (1 ml) rather than decapitation by guillotine.

The procedure utilised to measure CBF was initiated 2 - 5 minutes subsequent to drug administration (Section 7.3.2.2) using a modified method of Sakurada et al (1978). This time delay corresponded to the maximum hypertensive response to i.c. ET-1 previously described in Section 7.2.3.1. A ramped infusion over 30 seconds of [^{14}C]-iodoantipyrine (50 µCi in 1.5 ml saline) was administered whilst simultaneously collecting timed arterial

blood samples (a total of 14 - 18) from the femoral cannula onto pre-weighed filter papers. At the end of the infusion period and for reasons discussed previously the animal was killed either by decapitation or an overdose of anaesthetic. The drop rate and precise time of death were noted. The brain was then removed rapidly for freezing by immersion in 2-methyl butane at -45°C. Coronal brain sections were cut (20 μm thick) in a cryostat at -22°C, three in every ten sections were mounted on glass coverslips and immediately dried on a hot plate for autoradiographic processing. The next section in each cycle was stained with cresyl violet to facilitate neuroanatomical identification of brain nuclei. Autoradiographs were prepared by exposing the appropriate sections, with a set of plastic standards of known ^{14}C concentrations (44 - 1175 nCi/g) to X-ray film. Tissue optical density (OD) measurements from the autoradiographs were measured on a computer based densitometer with reference to the pre-calibrated standards. In the first series of experiments on anaesthetised animals the minimum tissue OD was measured in a general brain area from at least six sections in which this area was present. In the second study utilising conscious animals, tissue OD measurements were taken from at least six sections in which the discrete anatomical areas of interest could be accurately defined. The tissue OD measurements from both studies were converted with reference to the precalibrated standards into equivalent tracer concentrations.

The regional cerebral blood flow was then calculated by

solving the operational equation (Figure 7.8) derived originally by Kety (1951), using the concentration of the [^{14}C]-iodoantipyrine in the discrete regions of the brain, the blood-brain partition coefficient for the tracer (0.8), and the history of the tracer in arterial blood during the experimental period (Sakurada et al, 1978).

7.3.2.4 Statistical Analysis

The nature of the results obtained from the present study necessitated that the individual data sets for the haemodynamic studis were displayed for clarity. Where appropriate statistical analysis of these haemodynamic parameters was carried out on mean results using Repeated Measures Analysis of Variance with a Bonferroni correction included for multiple comparisons. The cerebral blood flow data was analysed using an unpaired students T-test.

7.3.3 Results

7.3.3.1 Effect of Intracisternal Administration of ET-1 on Blood Pressure and Heart Rate in the Anaesthetised Rat

The effect of intracisternal administration of ET-1 (0.03 nmols/rat) on blood pressure and heart rate in the anaesthetised rat is shown in Figure 7.9. Due to variation in the data these results are initially presented as individual values taken before and at the maximum blood pressure and heart rate response to intracisternal administration of ET-1. The haemodynamic response to intracisternal administration of ET-1

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

where Ci(T) equals the tissue concentration of the tracer at a time T after the introduction of the tracer into the circulation; λ equals the tissue : blood partition coefficient; C_A is the concentration of the tracer in the arterial blood; t equals the variable, time; and K equals a constant that incorporates within it the rate of blood flow in the tissue.

The constant K is defined as follows:

$$K = mF/W\lambda$$

where F/W equals the rate of blood flow per unit mass of tissue and m equals a constant between 0 and 1 that represents the extent to which diffusion equilibrium between blood and tissue is achieved during passage from the arterial to the venous capillaries.

Figure 7.8

Operational equation to determine cerebral blood flow using the [^{14}C]-iodoantipyrine autoradiographic technique

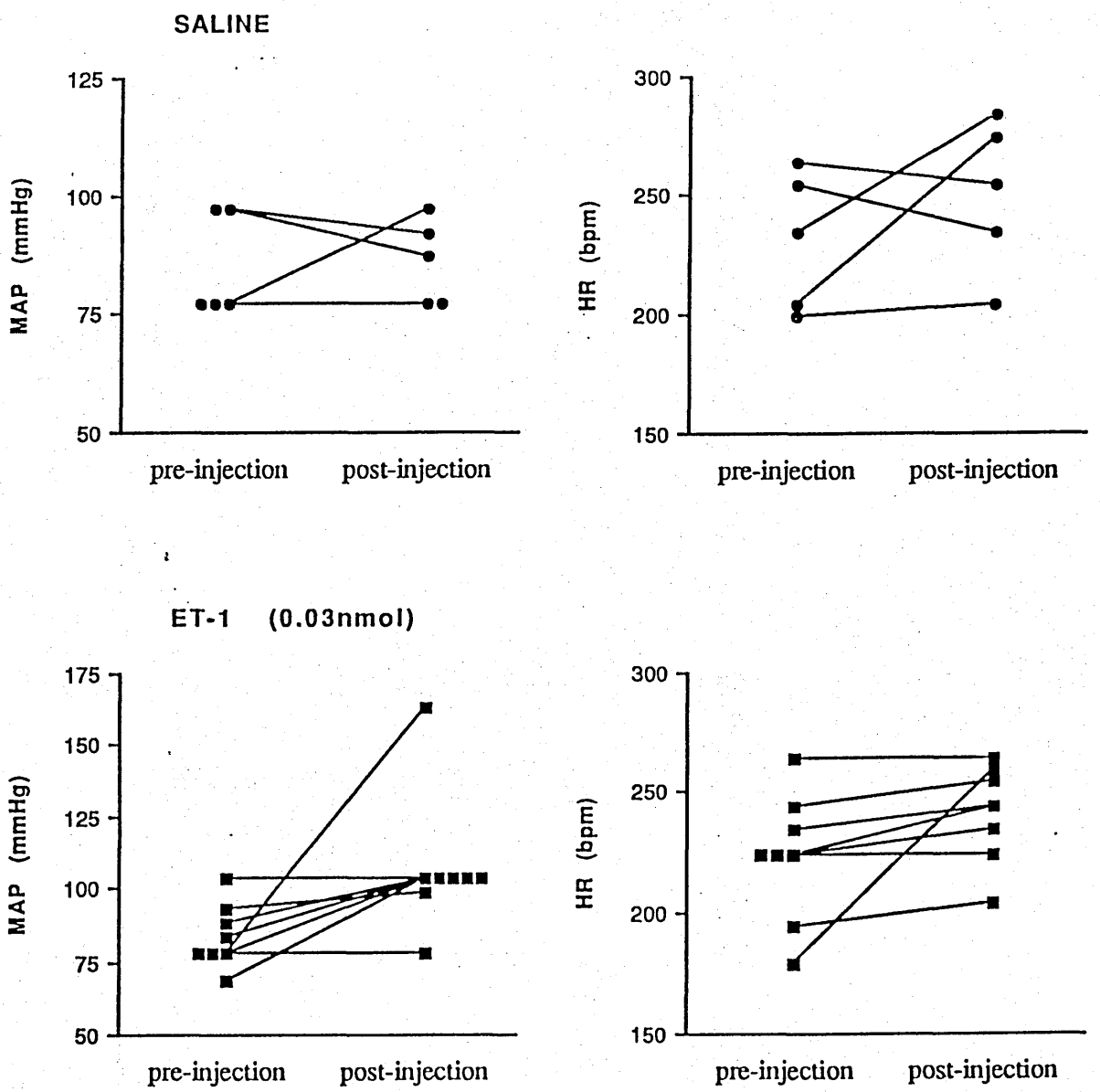


Figure 7.9

Individual blood pressure responses and corresponding heart rate values before and at the maximum response following intracisternal injection of ET-1 (0.03 nmol) or saline in the anaesthetised rat.

● Saline, n = 5

■ ET-1 (0.03 nmol), n = 8

(0.03 nmols), with one exception, did not appear to markedly vary from the control values (Figure 7.9). Indeed the maximum blood pressure and heart rate response for this group did not differ significantly from the pre-injection values (Table 7.4). Although, for obvious reasons a statistical test could not be applied, in the one animal that did respond to the peptide a maximum rise in blood pressure of 85 mmHg was observed two minutes after administration of ET-1 with no marked change in the corresponding heart rate (Table 7.4). This hypertensive event was similar to the haemodynamic effects of ET-1 previously demonstrated in the conscious rat (Section 7.2.3).

7.3.3.2 Effect of Intracisternal Administration of ET-1 on Cerebral Blood Flow in the Anaesthetised Rat

Regions of minimum cerebral blood flow (CBF) in three sites of neuroanatomical interest were examined in the animals described in Section 7.3.3.1. As shown in Table 7.5 the mean CBF values for the group of animals that did not respond to intracisternal administration of ET-1 were not significantly different from the control saline group. Although statistical analysis could not be carried out for obvious reasons, the CBF was markedly reduced, in all the brain regions shown in Table 7.5, in the animal which produced a hypertensive response to intracisternal administration of the peptide.

Table 7.4

Effect of Intracisternal ET-1 (0.03 nmol) on Blood Pressure (MAP) and Heart Rate (HR) in the Anaesthetised Rat

Drug/ Vehicle	MAP (mmHg)		HR (bpm)	
	Pre-injection	Post-injection	Pre-injection	Post-injection
Saline (n = 5)	83.0 ± 4.9	84.0 ± 4.0	227.0 ± 13.0	246 ± 14.4
ET-1 (n = 7)	82.1 ± 4.3	95.7 ± 3.5	223.6 ± 9.8	242.1 ± 5.33
ET-1 (n = 1)	75	160	190	200

Results are expressed where appropriate as mean values ± s.e.mean before and at the maximum response to intracisternal administration of saline or Peptide

Table 7.5

Effect of Intracisternal ET-1 (0.03 nmol) on Cerebral Blood Flow (CBF) in the Anaesthetised Rat

Region	CBF Values (ml $100g^{-1}$ min^{-1}) Saline (n = 5)	ET-1 (n = 7)	ET-1 (n = 1) Δ MAP = 85 mmHg
Lateral Medulla	89 ± 4	89 ± 6	10
Inferior Olives	125 ± 9	125 ± 7	11
Cerebellar White Matter	37 ± 7	31 ± 3	19

7.3.3.3 Effects of Intracisternal Administration of ET-1 on Blood Pressure and Heart Rate in the Conscious Rat

In the conscious rat, intracisternal administration of saline, as expected, had no effect on either blood pressure or heart rate as shown by the individual blood pressure and heart rate responses presented in Figure 7.10. In contrast, injection of ET-1 (0.03 nmols) by the same route, elicited differential effects on blood pressure. The individual haemodynamic responses before and at the maximum response to administration of the peptide are shown in Figure 7.10. It is clear from these results that in some, but not all animals, ET-1 (0.03 nmols) produced a distinct elevation in blood pressure. This hypertensive response had a latency of onset between 0.5 - 2 minutes reaching a maximum between 2-5 minutes. In this respect, the present results resemble those obtained in the previous haemodynamic study on conscious animals in Section 7.2.3. Increased activity of the animal prior to the hypertensive event was evident. However, since the animal was restrained the incidence of barrel-rolling could not be established. The blood pressure response has been subdivided into two groups based on whether or not a discernible (> 10 mmHg) increase in blood pressure occurred (Table 7.6). Statistical analysis was carried out to determine if the maximum (post-injection) blood pressure and heart rate responses of these subgroups differed significantly from their pre-injection values. In the group that 'responded' (Δ BP > 10 mmHg) to ET-1 (0.03 nmol), a significant increase in blood pressure was observed (Table 7.6). However, no significant alteration in the

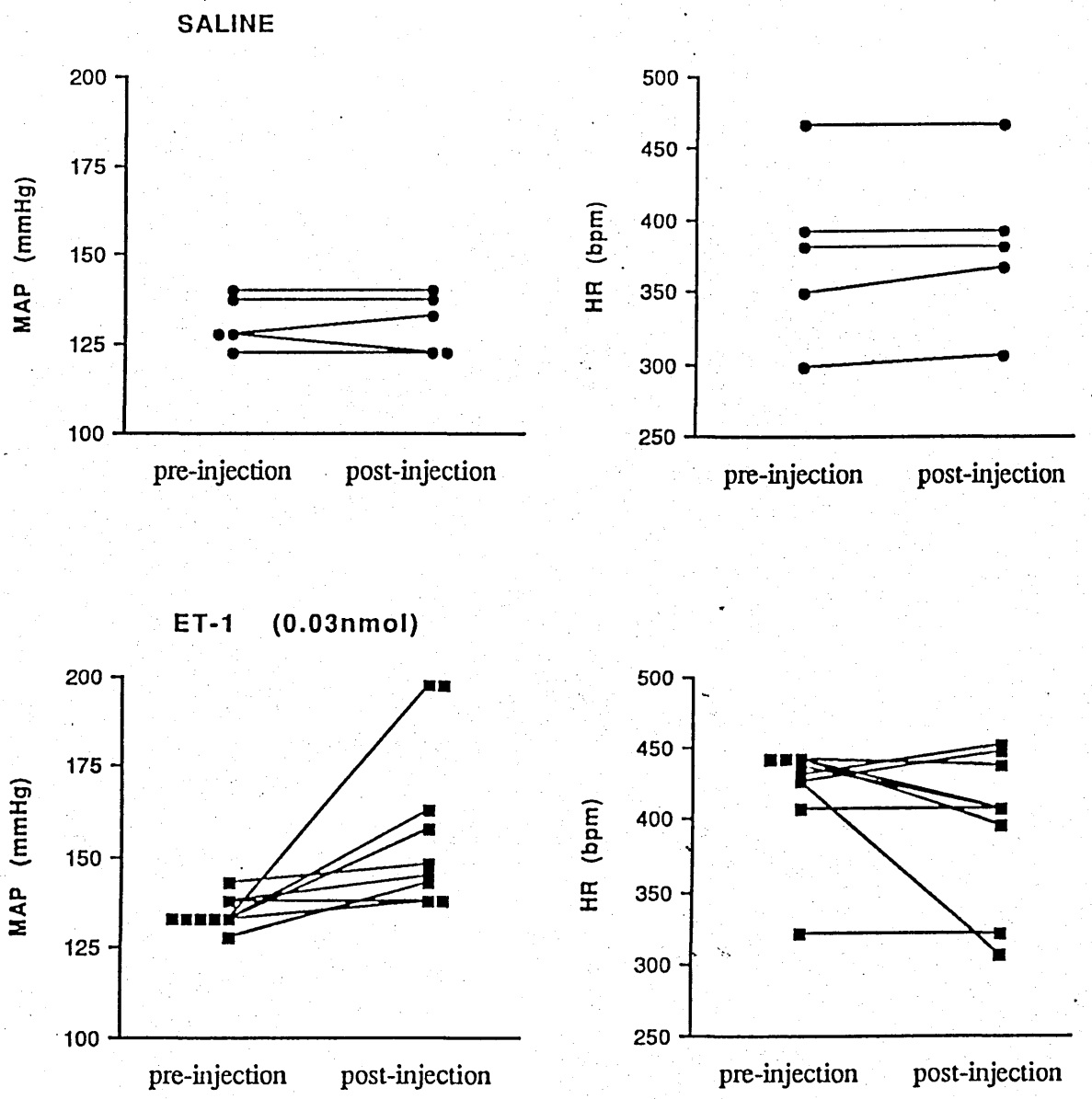


Figure 7.10

Individual blood pressure responses and corresponding heart rate values before and at the maximum response following intracisternal injection of ET-1 (0.03 nmol) or saline in the conscious rat.

O Saline, n = 5

■ ET-1 (0.03 nmol), n = 9

blood pressure response was observed either in the saline group or the subgroup of animals that were unresponsive (Δ BP < 10 mmHg) to the peptide (Table 7.6). No significant changes in heart rate were observed in any of the subgroups (Table 7.6).

7.3.3.4 Effects of Intracisternal Administration of ET-1 on Cerebral Blood Flow in the Conscious Rat

Analysis of the regional cerebral blood flow (CBF) from the conscious rats described above was carried out in three neuroanatomically defined regions of the medulla. The results revealed that CBF was consistently and significantly reduced, compared to the control (saline) group, in all medullary regions examined from the subgroup of animals where administration of the peptide produced the distinctive elevation in blood pressure (Table 7.7). Blood flows in the same regions from the subgroup of animals that did not 'respond' to i.c. ET-1 were not significantly different from the control group (Table 7.7). Representative autoradiographs from sections of the brains of two conscious rats are shown in Figure 7.11. A clear reduction in regional CBF is demonstrated in the section from an ET-1 treated animal that experienced a hypertensive event after administration of the peptide compared to a corresponding section from a control (saline)-treated animal.

Table 7.6

Effect of Intracisternal ET-1 (0.03 nmol) on Mean Arterial Pressure (MAP) and Heart Rate (HR) in the Conscious Rat

Drug/ Vehicle	MAP (mmHg)		HR (bpm)	
	Pre-injection	Post-injection	Pre-injection	Post-injection
Saline (n = 5)	128.0 ± 3.2	128.0 ± 3.6	371.0 ± 28	376.0 ± 26
ET-1 (BP < 10 mmHg) (n = 4)	135.0 ± 2:0	139.3 ± 2.5	423.8 ± 8.3	418.8 ± 11.3
ET-1 (BP > 10 mmHg) (n = 5)	129.0 ± 1.0	169.0 ± 11*	404.0 ± 22	369.0 ± 27

Results are expressed as mean values ± s.e.mean before and at the maximum response to intracisternal saline or peptide administration

* significant difference from saline control

* $p < 0.05$

Table 7.7

Effect of Intracisternal ET-1 (0.03 nmol) on Cerebral Blood Flow (CBF) in the Conscious Rat

Region	CBF Values (ml $100g^{-1}$ min^{-1})		
	Saline (n = 5)	ET-1 (n = 4) BP < 10 mmHg	ET-1 (n = 5) BP > 10 mmHg
Nucleus of the Solitary Tract	103 ± 4	116 ± 13	40 ± 13***
Caudal Ventrolateral Medulla	109 ± 4	130 ± 15	31 ± 20**
Rostral Ventrolateral Medulla	116 ± 7	127 ± 15	49 ± 25*

results are expressed as means s.e.mean

* significant difference from saline control

* $p < 0.05$ ** $p < 0.005$ *** $p < 0.001$

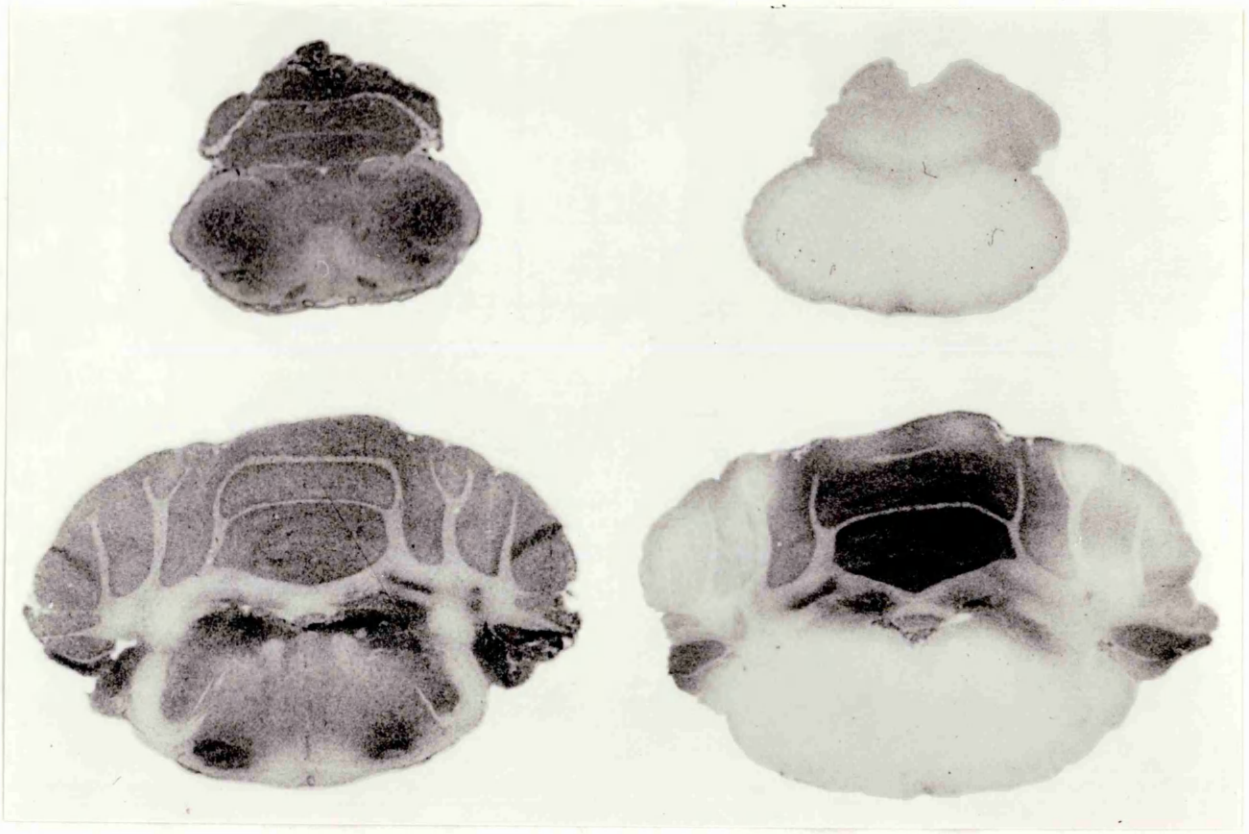


Figure 7.11

Representative autoradiographs showing a reduction in cerebral blood flow in sections from an animal that experienced hypertension after administration of ET-1 (0.03 nmols) compared to a corresponding section from a saline-treated animal.

a = control -- caudal medulla oblongata

b = ET-1 (0.03 nmols) -- caudal medulla oblongata

c = control - rostral medulla oblongata and pons

d = ET-1 (0.03 nmols) - rostral medulla oblongata and pons

7.3.4 Discussion

In conscious rats the marked elevation in arterial blood pressure, observed in some of the animals examined, subsequent to ET-1 administration, was associated with a pronounced reduction in cerebral blood flow (CBF). Indeed the extent and magnitude of this effect was far greater than any other peptide previously examined including neuropeptide Y (Tuor et al, 1987). The ability of this putative transmitter to modify CBF most likely results from a direct vasoconstrictor action on the cerebral vasculture but it could be a consequence of alterations in cerebral energy consumption. Evidence to support the former hypothesis derives from studies which described the constriction of isolated feline and canine basilar arteries in response to ET-1 (Yanagisawa et al, 1988a; Asano et al, 1989). In the same report, Asano et al (1989) also demonstrated, using angiographic techniques, a reduction in basilar artery diameter following intracisternal administration of ET-1 in anaesthetised dogs. Changes in the degree of vasoconstriction by ET-1 (10 pmols) could be observed in the absence of alterations in blood pressure. However this effect was dose-related and prominent but transient increases in blood pressure (40 mmHg) were observed at higher concentrations (100 pmol) of the peptide (Asano et al, 1989).

Thus, in the present study, the marked reduction in CBF most likely arises from intense cerebral vasoconstriction. It is proposed that the resultant ischemia and concomitant increase in PCO_2 levels, due to insufficient perfusion of the brain, would

then stimulate central chemosensitive regions (Dampney and Moon, 1980; Grady and Blaumanis, 1988). The dramatic increase in arterial pressure which ensues may therefore be an attempt (reflex) to maintain commensurate cerebral perfusion pressure. Neurones in the medulla oblongata, in particular the previously discussed C_1 cell group located in the ventrolateral regions, are thought to participate in the reflex responses to reduction in blood flow and oxygen perfusion of the brain. Indeed lesions of the C_1 area in the rabbit abrogate the prominent pressor response evoked by cerebral ischemia (Dampney and Moon, 1980). Moreover these neurones are ideal candidates as oxygen sensors since they contain abundant mitochondria, are in close proximity to capillaries and can influence spinal preganglionic vasomotor outflow (Milner et al, 1987; Ross et al, 1984b).

The event described above is similar to Cushing Response (Cushing, 1901). This phenomenon, characterised by a marked rise in arterial pressure and bradycardia, occurs when intracranial pressure approaches the levels of arterial pressure and prevents sufficient cerebral perfusion. Controversy surrounds the exact mechanism responsible for the stimulation of sympathetic nerve outflow and consequent increase in arterial pressure in the Cushing Response. However, ischemia with the associated increase in PCO_2, direct pressure and mechanical distortion of the brainstem have all been implicated (McGillicuddy et al, 1978; Grady and Blaumanis, 1988; Thompson and Malina, 1959).

Thus, the ET-1-induced cerebral vasoconstriction which has been proposed to result in the observed attenuation of CBF, may have a physiological or pathophysiological role in the control of cerebral blood flow. Indeed, the disturbances in the control of ET-1 production or metabolism could also be instrumental in the pathogenesis of cerebral vasospasm. This condition, in humans, can occur after subarachnoid haemorrhage secondary to rupture of an intracranial aneurysm. Although a number of agents such as arachidonic acid metabolites, noradrenaline and serotonin have been proposed as contributing factors, ET-1 may also participate (Asano et al, 1989).

A second mechanism by which ET-1 could alter cerebral blood flow is through modification of cerebral energy consumption - namely oxidative metabolism or glucose consumption. The relationship between CBF and cerebral metabolism emanates from the requirement of a continuous blood supply to maintain cerebral function. This is necessary since the brain only contains small amounts of energy generating substances. A number of agents have been shown to influence these parameters in parallel. Central administration of serotonin reduces cerebral functional activity as determined by oxygen and glucose utilisation as well as evoking a parallel reduction in CBF (Harper and MacKenzie, 1977). Opposite effects are elicited by vasoactive intestinal polypeptide (McCulloch and Edvinsson, 1980). This peptide increases cerebral energy requirements with an associated increase in CBF. Moreover, neuropeptide Y has been observed to reduce glucose utilisation in brainstem areas (Macrae et al,

1990, in press). In view of the lack of data concerning the alterations in cerebral metabolic activity following intracisternal administration of ET-1, a contribution from this source to the reduction in CBF cannot be excluded, although it is unlikely that a decrease in metabolic activity alone could account for the massive reductions in CBF observed.

The differences observed between conscious and anaesthetised rats in the frequency of the haemodynamic and parallel CBF response to intracisternal ET-1 may, reflect confounding effects of general anaesthesia on metabolic activity and CBF. Halothane anaesthesia elicits a reduction in total cerebral energy consumption associated with a reduction in consciousness (Shapiro et al, 1978). A direct action of halothane on cerebral blood vessels has also been suggested since vasodilation and not vasoconstriction, as expected from the metabolic data, has been reported (McCulloch, 1988). However, this proposed influence of anaesthesia on the central effects of ET-1 is not supported by the results obtained following intravenous administration of the peptide (Section 7.1.3). In that study, halothane anaesthesia altered the magnitude but not the frequency of the hypertensive response. Differences between the effects of halothane on the peripheral and cerebral circulation cannot be excluded. Perhaps the response to ET-1 varies with the activity of the animal. Thus, in the conscious animals, active head movement enabled the peptide to mix in the cerebral spinal fluid and reach the large and more distant blood vessels such as the basilar artery, on the

ventral surface of the brain. Indeed a time-lag (0.5 - 2 minutes) in the haemodynamic effects of intracisternal administration of ET-1 was observed in the present study implying a delay in reaching the site of action. This explanation however, does not account for the fact that some of the conscious animals did not respond to intracisternal administration of endothelin. In these animals the CBF resembled control values. This would suggest that distribution to the cerebral blood vessels was impeded in these animals. Shigeno et al (1989) have demonstrated a consistent reduction in basilar artery diameter following intracisternal administration of ET-1 in both cats and dogs under halothane anaesthesia. However, a large injectate volume (3 - 6 mls) was used in this study, possibly assisting distribution. Despite the fact that access to the cerebral spinal fluid (CSF) via the cannula was tested before each injection as described in Section 1.6 and the position of the cannula verified in certain animals at the end of the procedure it is feasible that there are problems associated with the technique in the present study. Since the completion of the studies contained within Chapter Seven, experiments have been undertaken to address the possibility that the inactivity of ET-1 (0.03 nmols) in some animals is a consequence of obstructed flow of the peptide through the cannula. Preliminary data from pilot experiments utilising the coadministration of unlabelled ET-1 (0.03 nmols) and [^{125}I]-radiolabelled ET-1 to trace the distribution of the peptides have demonstrated that in an animal that did not respond to the intracisternal coadministration, ET-1

binding sites could not be detected in the autoradiographs of brain tissue. This would suggest that in this case the passage of the peptide through the cannula was impaired. Moreover, ET-1 binding sites were observed throughout the brain tissue of an animal that responded with a hypertensive event to the coadministration regime. While further experiments are obviously needed to confirm this finding other explanations cannot be excluded. Interestingly, Wiklund et al (1988) reported a similar ambiguity in the effect of ET-1 on isolated femoral arteries. In this study, nine out of 21 preparations did not respond to the peptide. Although an interpretation was not advanced by these authors it is unlikely that restricted access would account for the unresponsiveness of some of the preparations.

The complications discussed above do not detract from the major finding of the current investigation that a reduction in cerebral blood flow is consistently associated with the hypertensive response following intracisternal administration of ET-1. Thus, ET-1 may have physiological relevance in the control of cerebral vascular tone as well as pathological importance in condiions such as cerebral vasospasm. However, further experiments are needed to resolve the apparent anomalies.

CHAPTER EIGHT

GENERAL DISCUSSION

Chapter Eight

General Discussion

The intention of this final chapter is to coalesce and abridge the findings of the individual studies contained within this thesis, which investigate the central cardiovascular activities of the recently discovered peptides, neuropeptide Y (NPY) and endothelin-1 (ET-1). The physiological and pathological implications of these findings will also be considered along with proposals for future work.

In Chapter One the putative role of NPY in the central haemodynamic regulatory mechanisms was examined in the anaesthetised rat, using a discrete microinjection technique. This permitted the investigation of two distinct regions of the medulla oblongata namely, the caudal ventrolateral medulla (CVLM) and the rostral ventrolateral medulla (RVLM). These areas both contain NPY-like immunoreactive cell bodies as well as terminals and are considered pivotal in the tonic and reflex control of the cardiovascular system (Harfstrand et al, 1987a). NPY (25 pmol and 50 pmol) injected into the CVLM of the anaesthetised rat evoked a dose-related hypotension which, it is proposed, may be associated with the stimulation of a group of noradrenergic vasodepressor (A_1) neurones located in this region. NPY also elicited a pronounced fall in heart rate, which was not dose-related. One explanation for this finding may be the close proximity and possible involvement of the nucleus ambiguus, an area with cardioinhibitory vagal projections (Ciriello and

Calaresu, 1980c; Macrae and Reid, 1988). This central vasodepressor effect of NPY in the CVLM is in accordance with another study which described a hypotensive response following intracisternal administration of this peptide (Fuxe et al, 1983). Since administration by this latter route concentrates the peptide around the medulla oblongata, it is possible that the A_1 neurones in the CVLM contribute to this response following intracisternal administration of NPY.

Although in the present study, injection of NPY (25 and 50 pmol) into the RVLM did not elicit any significant haemodynamic response, spinally projecting NPY-containing neurones in this area have been purported to be involved in the haemodynamic response elicited from A_1 neurones. This conjecture has arisen from pharmacological and neuroanatomical studies which have indicated that the sympathoexcitatory neurones in the RVLM receive a tonic inhibitory input from the CVLM (Granata et al, 1986; Chan et al, 1986). Moreover when neurones in the CVLM are inhibited (or neurones in the RVLM directly stimulated) an increase in both blood pressure and the release of NPY-like immunoreactivity from the spinal cord ensue (Pilowsky et al, 1987; Chalmers et al, 1986). Whether NPY is the endogenous transmitter responsible for stimulating the sympathetic preganglionic neurones and consequently elevating blood pressure is unclear, since intrathecal injection of NPY has been shown to evoke a vasodepressor response (Westfall et al, 1988). It is apparent that further work is needed before the potential of NPY to regulate blood pressure and heart rate in these regions is

fully explored. This research is hindered by the absence of a specific NPY antagonist.

In addition to the proposed role for NPY in the normal functioning of central cardiovascular dynamics, supported by the studies, dysfunction of mechanisms regulating this peptide may be involved in the development and/or maintenance of hypertension. Indeed radioimmunoassay studies by Maccarrone and Jarrott (1985) have shown that lower levels of this vasodepressor peptide are present in the brainstem and spinal cord of spontaneously hypertensive rats (SHR). Moreover the hypotension elicited by intrathecal injection of NPY is attenuated in SHR rats (Westfall et al, 1988). Other alterations in NPY functioning have been observed in the SHR rat including increased binding sites in the area postrema (Nakajima et al, 1987). It is of considerable interest that many of the central neuronal systems containing catecholamines, which are considered to be of fundamental importance in the central regulation of blood pressure and whose function is altered in experimental hypertension, also contain NPY (Chalmers, 1975). NPY is costored, for instance, with noradrenaline in the A_1 cell group of the CVLM and in the more rostrally located C_1 adrenergic cell group (Harfstrand et al, 1987a). The functional implications of this coexistence with regard to haemodynamic regulation, was investigated in Chapter Four where the possible interaction between NPY receptors and α_2-adrenoceptors was examined. The involvement of α_2-adrenoceptors in the central neural regulation of the

cardiovascular system has been recognised and it has been proposed that the catecholamines, adrenaline and noradrenaline are the endogenous ligands for these brainstem α_2-adrenoceptors (Unnerstall et al, 1984; Hausler, 1982). In Chapter Three it was previously demonstrated that the α_2-adrenoceptor agonist clonidine (10 and 20 nmol) produced hypotension and bradycardia following microinjection into both the CVLM and RVLM. Coadministration of NPY (25 or 50 pmol) together with a submaximal dose of clonidine (10 nmol) into the CVLM, produced a hypotensive response which was similar to the sum of their individual blood pressure responses. This indicated that no interaction occurred between the separate blood pressure responses evoked by the agents through their respective receptors. A similar lack of interaction was observed in the RVLM. NPY (25 pmol) which alone had no effect on blood pressure did not alter the haemodynamic effects of clonidine. It is interesting that in the CVLM although the hypotensive response to clonidine was not affected by NPY, simultaneous injection of NPY (25 pmol) and clonidine (10 nmol) produced a bradycardia which was less than the sum of the individual heart rate responses. The functional significance of this apparent antagonistic interaction remains to be determined. However, the general absence of functional interaction between α_2-adrenoceptors and NPY receptors, with regard to the blood pressure response, differed from a report by Carter et al (1985) who demonstrated that the hypotensive response to 20 nmol noradrenaline in the nucleus of the solitary tract (NTS), was significantly modified

by both prior and simultaneous injection of an ineffective dose (47 fmol) of NPY. The reason for the differences between studies is not clear since colocalisation of NPY and catecholamines has been observed in the neuronal groups of the ventrolateral aspects of the medulla as well as in the NTS. However, colocalisation may not always denote a probable functional interaction. Indeed, the classical transmitter somatostatin and NPY are costored in the striatum but do not appear to interact in the regulation of dopamine turnover when coadministered, implying a separate mechanism of action (Kowall et al, 1985). In some regions colocalisation may reflect a provision for separate functions which are required or evoked under different conditions. In sympathetic neurones in the periphery where NPY and noradrenaline are costored, differential release of these agents has been observed lending support to this postulate. Low frequency stimulation preferentially releases noradrenaline whereas high frequency stimulation is needed to release both noradrenaline and NPY (Lundberg et al, 1986). Thus, it has been proposed that NPY may only be released under conditions of high frequency stimulation such as 'stress' to support the classical transmitter and improve transmitter economy.

The molecular mechanism involved in the hypotensive action of NPY and possible interactions with adrenoceptors were examined in Chapters Five and Six. Since NPY was previously shown to modulate cAMP levels in cerebral blood vessels and cortex (Fredholm et al, 1985; Westlind-Danielsson, 1987), experiments

were devised to test whether this second messenger system could also participate in the central cardiovascular activities of NPY observed in the medulla. A significant reduction of forskolin-stimulated but not basal cAMP levels was demonstrated with 10^{-6}M NPY in slices of the rat medulla oblongata as well as in the cortex. In addition, the ability of NPY to inhibit forskolin-stimulated cAMP levels in medullary slices was abolished following intracisternal pre-treatment with pertussis toxin (2 or 5 μg). This toxin inactivates a family of inhibitory G-proteins which are known to mediate the reduction of adenylate cyclase activity and consequently a reduction in cAMP levels. It is therefore postulated that the reduction in cAMP levels produced by NPY in the medulla is regulated through an inhibitory G-protein.

Consistent with the hypothesis that the hypotensive effect of NPY in the medulla is mediated via a reduction in adenylate cyclase activity and cAMP levels, a very recent publication by Fuxe et al (1989) demonstrates that intraventricular pretreatment with PTX inhibits the hypotensive and bradycardic effects of NPY injected into the lateral ventricles. Interestingly in this study the hypotensive but not the bradycardic effect of clonidine was also attenuated by PTX pretreatment. Although the central hypotensive properties of clonidine were confirmed in the current studies, this α_2-adrenoceptor agonist was not observed to reduce forskolin-stimulated or basal cAMP levels in slices of rat medulla oblongata. It is noteworthy that while the inhibitory coupling of α_2-adrenoceptors to adenylate cyclase is established

in the peripheral nervous system, the situation is less clear and results more variable in the central nervous system. Thus, clonidine has been observed to either decrease, increase or have no effect on cAMP levels in central regions (Duman and Enna, 1986; Nakamichi et al, 1987; Etgen and Petitti, 1987). Differences in concentrations of the agonist used in these studies and variations in receptor populations between regions may contribute to these variations in response.

Moxonidine, another centrally acting α_2-adrenoceptor agonist with hypotensive properties, was observed to reduce forskolin-stimulated cAMP levels in medullary slices. In addition to being a full agonist at α_2-adrenoceptors (Clonidine is a partial agonist) another reason for its disparate effects from clonidine may be a consequence of its higher presynaptic α_2-adrenoceptor selectivity. Although haemodynamic interactions between NPY and α_2-adrenoceptors were generally absent in the ventrolateral medulla, a functional interaction has been reported between these receptors in the NTS (Carter et al, 1985). This receptor interaction may exist at the level of the recognition site as suggested by Fuxe et al (1987b) or at post-receptor processes. Evidence has been presented in Chapter Six which indicates that an interaction between the α_2-adrenoceptor agonist moxonidine and NPY can be detected at the second messenger level. Thus, the previously discussed individual inhibitory effects of these agents on forskolin-stimulated cAMP levels in the medulla oblongata were attenuated when these agents

were coadministered, suggesting an interaction between α_2-adrenoceptors and NPY receptors can be distinguished at the level of the second messenger system. In vivo microinjection studies on the haemodynamic effects of NPY and moxonidine alone and in combination are necessary to establish if an interaction is also evident at the circulation level. The cardiovascular effects of catecholamines which are found costored with NPY in medullary neurones may also be mediated through β-adrenoceptors located in this region (Stone and U'Prichard, 1981). However, an interaction between β-adrenoceptors and NPY receptors at the adenylate cyclase level was not evident in slices of medulla oblongata. In vivo microinjection studies observing the haemodynamic responses of these agents alone and when coadministered are proposed. Moreover, development of a method to microinject into the medullary regions of conscious rats would circumvent the possible confounding effects of anaesthesia and would therefore be beneficial in such studies.

The haemodynamic actions of another peptide, endothelin-1 (ET-1), a member of the recently identified endothelin family was examined in Chapter Seven. Intracisternal administration of ET-1 (0.01 -0.03 nmols) in the conscious rat evoked a distinctive response consisting of a dramatic elevation in blood pressure, accompanied in some cases by intense motor activity (barrel-rolling), but no consistent alteration in heart rate. This haemodynamic profile, following intracisternal ET-1 administration, occurred at lower concentrations than those needed to elicit a response in the periphery and also differed

qualitatively from the biphasic haemodynamic response produced following intravenous ET-1 (0.1 -1 nmol) administration. This data would suggest the response to intracisternal administration of ET-1 is central in origin and not due to leakage of the peptide into the periphery. An autoradiographic approach was utilised to elucidate the possible sites and mechanisms of the central action of ET-1. The distinctive cardiovascular response (reminiscent of Cushing Syndrome) and the now rapidly accumulating publications which assert a vasoconstrictor action of ET-1, suggest that the dramatic hypertensive event may be mediated via an action on cerebral blood vessels rather than an action in the brain parenchyma.

This hypothesis was investigated in the third section of Chapter Seven using [^{14}C] iodoantipyrene autoradiography for measurement of regional cerebral blood flow (CBF). The data obtained, indicated that the prominent increase in blood pressure following intracisternal administration of ET-1 was in all cases accompanied by a decrease in CBF in the brain stem to ischaemic levels. Moreover, this reduction in brainstem CBF was greater in magnitude than that previously observed with other putative endogenous transmitters. This data supports an angiographic study which was published subsequent to the present investigation and described cerebral vasoconstriction following intracisternal administration of ET-1 in dogs (Asano et al, 1989). Aberrations were in evidence in the present study. The hypertensive event was not present in all animals (conscious or anaesthetised)

investigated, moreover in those animals that did not respond to intracisternal endothelin-1 CBF was comparable to control values. The frequency of the hypertensive response in these animals was apparently reduced in the presence of anaesthesia. While an effect of anaesthesia per se on the frequency of the response to ET-1 is possible (though unlikely considering anaesthesia does not alter the frequency of the peripheral response), further work is required to resolve the 'non-response' of some animals to intracisternal administration of the peptide. Despite these anomalies considerable evidence has been presented which purports an involvement of ET-1 in centrally mediated haemodynamics and cerebral vascular function. It has also been speculated that a breakdown of the regulatory mechanisms of ET-1 production could lead to various pathological conditions such as cerebral vasospasm. Although there is no direct evidence to confirm this supposition, the potent and prolonged nature of the contracture observed following intracisternal administration of ET-1 in the present study and in the angiographic study by Asano et al (1989) suggest that this peptide may contribute to chronic cerebral vasospasm and its sequelae. Moreover that cerebral vasospasm can occur after subarachnoid haemorrhage secondary to rupture of an intracranial aneurysm and that the expression of ET-1 is stimulated in the presence of factors which are present at the site of endothelial injury (thrombin, TGF-F, shear stress) indicate an association of this peptide with the pathogenesis of vasospasm. In addition to its direct vasoconstricting action ET-1 may also participate in neural cardiovascular regulatory

mechanisms acting as a neuropeptide or neuromodulator. This putative role of ET-1 remains to be established. However Asano et al (1989) have shown that a low dose of ET-1 (10 pmol i.c.) evoked angiographic vasospasm with no elevation in blood pressure. This indicates that the first line of action of this peptide in the modulation of cerebral circulation in vivo appears to be the cerebral vessels.

In conclusion, both NPY and ET-1 have been observed to alter haemodynamic parameters following central administration in the rat. Some insight into the possible mechanisms of these actions have been presented. However, as discussed, further work is required to elucidate fully the potential role of both peptides in central cardiovascular regulatory mechanisms. The development of specific antagonists to NPY and ET-1 would undoubtedly expediate this research.

PRESENTATIONS AND PUBLICATIONS RESULTING FROM WORK UNDERTAKEN FOR THIS THESIS

McAuley, M.A., Macrae, I.M., Elfellah, M.S. and Reid, J.L. (1989). The effect of NPY on cAMP levels in slices of rat medulla. Proc. Br. J. Pharmacol. 96: 345P.

McAuley, M.A., Macrae, I.M. and Reid, J.L. (1988). Central cardiovascular effects of neuropeptide Y and clonidine in the rat. Proc. Br. J. Pharmacol. 94: 434P.

McAuley, M.A., Macrae, I.M. and Reid, J.L. (1988). The cardiovascular actions of clonidine and neuropeptide Y in the ventrolateral medulla of the rat. Br. J. Pharmacol. 97: 1067-1074.

McAuley, M.A., Macrae, I.M. and Reid, J.L. (1989). Haemodynamic responses to endothelin after peripheral and central administration in the conscious rat. Proc. Br. J. Pharmacol. 98: 709P.

Reid, J.L., Hamilton, C.A., Macrae, I.M., Yakabu, M.A. and McAuley, M.A. (1988). Central adrenergic regulation of blood pressure: Transmitter and receptor heterogeneity. In: Progress in catecholamine research; Central Aspects. A.R. Liss, Inc.: 311-315.

Robinson, M.J., Macrae, I.M., McAuley, M.A., Teasdale, J.M., McCulloch, J. (1989). Brainstem ischaemia induced by endothelin - A vasoconstrictor peptide. British J. Surgery (in press).

REFERENCES

Adrian, T.E., Allen, J.M., Bloom, S.R., Ghatei, M.A., Rossor, M.N., Roberts, G.W., Crow, T.J., Tatemoto, K. and Polak, J.M. (1983). Neuropeptide Y distribution in human brain. Nature 306: 584-586.

Aghajanian, G.K. and Wang, Y-Y. (1986). Pertussis toxin blocks the outward currents evoked by opiate and α_2-agonists in locus coeruleus neurones. Brain Res. 371: 390-394.

Agnati, L.F., Fuxe, K., Benfenati, F., Battistini, N., Harstrand, A., Tatemoto, K., Hokfelt, T., Mutt, V. (1983). Neuropeptide Y in vitro selectively increases the number of α_2-adrenergic binding sites in membranes of the medulla oblongata of the rat. Acta Physiol. Scand. 118: 293-295.

Alexander, R.S. (1946). Tonic and reflex functions of medullary sympathetic cardiovascular centers. J. Neurophysiol. 9: 205-217.

Al-Gadi, M. and Hill, S.J. (1985). Characterisation of histamine receptors mediating the stimulation of cyclic AMP accumulation in rabbit cerebral cortical slices. Br. J. Pharmacol. 85: 877-888.

Allen, J.M., Adrian, T.E., Polak, J.M. and Bloom, S.R. (1983b). Neuropeptide Y (NPY) in the adrenal gland. J. Auton. Nerv. Syst. 9: 559-563.

Allen, Y.S., Adrian, T.E., Allen, J.M., Tatemoto, K., Crow, T.J., Bloom, S.R. and Polak, J.M. (1983a). Neuropeptide Y distribution in the rat brain. Science 221: 877-879.

Allen, J.M., Gibson, S.J., Adrian, T.E., Polak, J.M. and Bloom, S.R. (1984). Neuropeptide Y in human spinal cord. Brain Res. 308: 145-148.

Allen, J.M., Raine, A.E.G., Ledingham, J.G.G. and Bloom, S.R. (1985). Neuropeptide Y: A novel renal peptide with vasoconstrictor and natriuretic activity. Clin. Sci. 68: 373-377.

Altura, B.M. (1970). Significance of amino acid residues in vasopressin on contraction in vascular muscle. Am. J. Physiol. 219: 222-229.

Amendt, K., Czachurski, J., Dembowsky, K. and Seller, H. (1979). Bulbospinal projections to the intermediolateral cell column; a neuroantomical study. J. Auton. Nerv. Syst. 1: 103-117.

Andrade, R. and Aghajanian, G.K. (1985). Opiate and α_2-adrenoceptor-induced hyperpolarisation of locus coeruleus neurons in brain slices: reversal by cyclic adenosine 3'5'-monophosphate analogues. J. Neurosci. 5: 2359-2364.

Andrezik, J.A., Chan-Palay, V. and Palay, S.L. (1981). The nucleus paragigantocellularis lateralis in the rat: demonstration of afferents by the retrograde transport of horseradish peroxidase. Anat. Embryol. 161: 373-390.

Antonaccio, M.J. and Halley, J. (1977). Clonidine hypotension: lack of effect of bilateral lesions of the nucleus solitary tract in anaesthetised cats. Neuropharmacol. 16: 431-433.

Armah, I.B. (1986). Moxonidine (BE 5895) induces its hypotensive action via selective stimulation of presynaptic alpha$_2$-adrenoceptors. Naunyn Schmiedeberg's Arch. Pharmacol. 332: R76.

Armah, I.B. (1987). Contribution of presynaptic α_2-adrenoceptors stimulation to the antihypertensive action of moxonidine. J. Cardiovasc. Pharmacol. 10 (Suppl. 4): S81-S83.

Armah, I.B. and Stenzel, W. (1981). BE 5895, a new clonidine-type antihypertensive aminopyrimidine derivative. Naunyn Schmiedeberg's Arch. Pharmacol. 316: R42.

Armstrong, D.M., Ross, C.A., Pickel, V.P., Jon, T.H. and Reis, D.J. (1982). Distribution of dopamine-, noradrenaline-, and adrenaline-containing cell bodies in the rat medulla oblongata: Demonstrated by immunohistochemical localisation of catecholamine biosynthetic enzymes. J. Comp. Neurol. 212: 173-187.

Asano, T., Ikegaki, I., Suzuki, Y., Satoh, S-I. and Shibuya, M. (1989). Endothelin and the production of cerebral vasospasm in dogs. Biochem. Biophys. Res. Commun. 159 (No.3): 1345-1351.

Atkinson, J., Lambas-senas, L., Parker, M., Boillat, N., Luthi, P., Sonnay, M., Seccia, M. and Renaud, B. (1986). Chronic clonidine treatment and its withdrawal: Effects on blood pressure and catecholamine synthesising enzymes in brainstem nuclei. Eur. J. Pharmacol. 121: 97-106.

Atkinson, J., Sonnay, M. and Boillat, N. (1984). Changes in central monoaminergic function during chronic treatment with clonidine in the spontaneously hypertensive rat. Eur. J. Pharmacol. 106: 613-617.

Atlas, D. and Burstein, Y. (1984). Isolation and partial purification of a clonidine displacing endogenous brain substance. Eur. J. Biochem. 144: 287-293.

Auguet, M., Delaflotte, S., Chabrier, P-E., Parotzky, E., Clostre, F. and Braquet, P. (1988). Endothelin and Ca^{2+} agonist Bay K8644: different vasoconstrictive properties. Biochem. Biophys. Res. Commun. 158 (1): 186-192.

Bahner, U., Palkovits, M., Geiger, H., Schmid, G. and Heidland (1989). Adenylate cyclase system in microdissected brain areas of normotensive and spontaneously hypertensive rats. J. Chem. Neuroanat. 2: 45-55.

Bai, F.L., Yamano, Y., Shiotani, P., Emson, P.C., Smith, A.D., Powell, J.R. and Tohyama, M. (1985). An arcuato-paraventricular and dorsomedial hypothalamic neuropeptide Y-containing system which lacks noradrenaline in the rat. Brain Res. 331: 172-175.

Baraban, J.M., Snyder, S.H. and Alger, B.E. (1985). Protein kinase C regulated ionic conductances in hippocampal pyramidal neurons: electrophysiological effects of phorbol esters. Proc. Natl. Acad. Sci. 82: 2538-2542.

Benarroch, E.E., Granata, A.R., Giuliano, R. and Reis, D.J. (1986). Neurons of the C_1 area of rostral ventrolateral medulla mediate nucleus tractus solitarii hypertension. Hypertension 8 (Suppl. I): I-56-I-60.

Berhgerhausen, J. (1985). Moxonidine (BE 5895), a full agonist at human platelet α_2-adrenoceptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 325 (Suppl.): R80.

Bernard, C. (1863). Lecons sur la physiologie et la pathologie du systeme nerveux. Paris, Bailliere, Vol. 1.

Berridge, M.J. and Irvine, R.F. (1989). Inositol phosphates and cell signalling. Nature 341: 197-205.

Berrie, C.P., Birdsall, N.J.M., Burgen, A.S.V. and Hume, E.C. (1979). Guanine nucleotides modulate muscarinic receptor binding in the heart. Biochem. Biophys. Res. Commun. 87: 1000-1005.

Blessing, W.W., Costa, M., Furness, J.B. and West, M.J. (1981b). Projection from A_1 neurons towards the nucleus tractus solitarius in rabbits. Cell Tissue Res. 220: 27-40.

Blessing, W.W., Goodchild, A.K., Dampney, R.A.L. and Chalmers, J.P. (1981a). Cell groups in the lower brainstem of the rabbit projecting to the spinal cord, with special reference to catecholamine-containing neurons. Brain Res. 221: 35-55.

Blessing, W.W., Hedger, S.C., Joh, T.H. and Willoughby, J.O. (1987a). Neurones in the area postrema are the only catecholamine-synthesising cells in the medulla or pons with projections to the rostral ventrolateral medulla (C_1-area) in the rabbit. Brain Res. 419: 336-340.

Blessing, W.W., Howe, P.R.C., Joh, T.H., Oliver, J.R. and Willoughby, J.O. (1986). Distribution of tyrosine hydroxylase and neuropeptide Y-like immunoreactive neurons in rabbit medulla oblongata, with attention to colocalisation studies, presumptive adrenaline-synthesising perikarya, and vagal preganglionic cells. J. Comp. Neurol. 248: 285-300.

Blessing, W.W., Oliver, J.R., Hodgson, A.H., Joh, T.H., Willoughby, J.O. (1987b). Neuropeptide Y-like immunoreactive C_1 neurons in the rostral ventrolateral medulla of the rabbit project to sympathetic preganglionic neurons in the spinal cord. J. Auton. Nerv. Syst. 18: 121-129.

Blessing, W.W. and Reis, D.J. (1982). Inhibitory cardiovascular function of neurons in the caudal ventrolateral medulla of the rabbit: relationship to the area containing A_1 noradrenergic cells. Brain Res. 253: 161-171.

Blessing, W.W., Sved, A.F. and Reis, D.J. (1982). Destruction of noradrenergic neurones in rabbit brainstem elevates plasma vasopressin, causing hypertension. Science 217: 661-663.

Blessing, W.W. and Willoughby, J.O. (1985). Excitation of neuronal function in rabbit caudal ventrolateral medulla elevates plasma vasopressin. Neurosci. Lett. 58: 189-194.

Bloom, F.E. (1979). Contrasting principles of synaptic physiology, peptidergic and non-peptidergic neurones. In: Hokfelt, T. and Luft, R. (eds.). Central regulation of the endocrine system. New Foundation Symposium, 42, Plenum Press, New York: 173-187.

Borkowski, K.R. and Finch, L.J. (1979). A comparison of the cardiovascular effects of centrally administered clonidine and adrenaline in the anaesthetised rat. J. Pharm. Pharmac. 31: 16-19.

Bousquet, P., Feldman, J., Bloch, R. and Schwartz, J. (1981). The nucleus reticularis lateralis: A region highly sensitive to clonidine. Eur. J. of Pharmacol. 69: 389-392.

Bousquet, P., Feldman, J. and Schwartz, J. (1984). Central cardiovascular effects of alpha adrenergic drugs: differences between catecholamines and imidazolines. J. Pharmacol. and Exper. Ther. 230: 232-236.

Bousquet, P. and Schwartz, J. (1983). Alpha-adrenergic drugs. Pharmacological tools for the study of the central vasomotor control. Biochem. Pharmacol. 32: 1459-1465.

Brody, M.J. (1986). Central nervous system mechanisms of arterial pressure regulation. Fed. Proc. 45: 2700-2706.

Brody, M.J., Haywood, J.R., Touw, K.B. (1980). Neural mechanisms in hypertension. Ann. Rev. Physiol. 42: 441-453.

Bucholz, R.A. and Nathan, M.A. (1984). Chronic lability of the arterial blood pressure produced by electrolytic lesions of the nucleus tractus solitarii in the rat. Circ. Res. 54: 227-238.

Burns, D.L. (1988). Subunit, structure and enzymic activity of pertussis toxin. Microbiological Sciences 5 (No.9): 285-287.

Burnstock, G., Hokfelt, T., Gershon, M.D., Iversen, L.I., Kosterlitz, H.W. and Szurszewski, J.H. (1979). Non-adrenergic, non-cholinergic autonomic neurotransmission mechanisms. Neurosci. Res. Program. Bull. 17: 377-519.

Bylund, D.B. and Snyder, S.H. (1976). Beta adrenergic receptor binding in membrane preparations from mammalian brain. Mol. Pharmacol. 12: 568-580.

Cabot, J.B. and Guyenet, P.G. (1981). Inhibition of sympathetic preganglionic neurones by catecholamines and clonidine: mediation by an alpha-adrenergic receptor. J. Neurosci. 1: 908-917.

Calaresu, F.R. Faiers, A.A. and Mogenson, G.J. (1975). Central neural regulation of heart and blood vessels in mammals. Prog. Neurobiol. 5: 1-35.

Carlsson, A., Lindqvist, M., Magnusson, T. and Waldeck, B. (1958). On the presence of 3-Hydroxytyramine in brain. Science 127: 471.

Carter, D.A. and Lightman, S.L. (1986). Comparative distribution and cardiovascular actions of substance P and substance K within the nucleus tractus solitarius of rat. Neuropeptides 6: 425-436.

Carter, D.A., Vallejo, M. and Lightman, S.L. (1985). Cardiovascular effects on neuropeptide Y in the nucleus tractus solitarius of rats: Relationship with noradrenaline and vasopressin. Peptides 6: 421-425.

Chalmers, J.P. (1975). Brain amines and models of experimental hypertension. Circ. Res. 36: 469-479.

Chalmers, J., Kapoor, V., Mills, E., Minson, J., Morris, M., Pilowsky, P. and West, M. (1986). Do pressor neurons in the ventrolateral medulla release amines and neuropeptides? Can. J. Physiol. Pharmacol. 65: 1598-1604.

Chan, S.H.H., Chan, J.Y.H. and Ong, B.T. (1986). Anatomic connections between nucleus reticularis rostroventrolateralis and some medullary cardiovascular sites in the rat. Neurosci. Lett. 71: 277-282.

Chang, R.S.L., Lotti, V.J., Chen, T.B., Cerino, D.J. and Kling, P.J. (1985). Neuropeptide Y (NPY) binding sites in rat brain labelled with I^{125}-bolton-Hunter NPY: Comparative potencies of various polypeptides on brain NPY binding and biological responses in the rat vas deferens. Life Sciences 37: 2111-2122.

Chen, X., Henderson, K., Beinfeld, M.C. and Westfall, T.C. (1988). Alterations in blood pressure of normotensive and hypertensive rats following intrathecal injections of neuropeptide Y. J. Cardiovasc. Pharmacol. 12: 473-478.

Cherie Millar, B., Piper, H.M. and McDermott, B. (1988). The antiadrenergic effect of neuropeptide Y on the ventricular cardiomyocyte. Naunyn-Schmiedeberg's Arch. Pharmacol. 338: 426-429.

Chronwall, B.M., Chase, T.N. and O'Donohue, T.L. (1984). Coexistence of neuropeptide Y and somatostatin in human cortical and rat hypothalamic neurons. Neurosci. Lett. 52: 213-217.

Chronwall, B.M., DiMaggio, D.A., Massari, V.J., Pickel, D.A., Ruggiero, D.A. and O'Donohue, T.L. (1985). The anatomy of neuropeptide Y containing neurons in rat brain. Neurosci. 15: 1159-1181.

Ciriello, J. and Calaresu, F.R. (1980a). Role of paraventricular and supraoptic nuclei in central cardiovascular regulation in the rat. Am. J. Physiol. 239: R137-R142.

Ciriello, J. and Calaresu, F.R. (1980b). Autoradiographic study of ascending projections from cardiovascular sites in the nucleus tractus solitarii in the cat. Brain Res. 186: 448-453.

Ciriello, J. and Calaresu, F.R. (1980c). Distribution of vagal cardioinhibitory neurons in the medulla of the cat. Am. J. Physiol. 238: R57-R64.

Ciriello, J. and Caverson, M.M. (1986). Bidirectional cardiovascular connections between ventrolateral medulla and nucleus of the solitary tract. Brain Res. 367: 273-281.

Cockcroft, S. and Gomperts, B.D. (1985). Role of guanine nucleotide binding protein in the activation of polyphosphoinositide phosphodiesterase. Nature 314: 534-536.

Cohn, M.L. and Cohn, M. (1975). 'Barrel rotation' induced by somatostatin in the non-lesioned rat. Brain Res. 96: 138-141.

Colmers, W.F., Lukowiak, K. and Pittman, Q.J. (1987). Presynaptic action of neuropeptide Y in area CA_1 of the rat hippocampal slice. J. Physiol. 383: 285-299.

Connor, H.E. and Finch, L. (1981). Postsynaptic spinal alpha-adrenoceptors mediate effects of intrathecal clonidine. Eur. J. Pharmacol. 76: 97-100.

Cowley, A.W., Liard, J.R. and Guyton, A.C. (1973). Role of the baroreceptor reflex in daily control of arterial blood pressure and other variables in dogs. Circ. Res. 32: 564-576.

Cushing, H. (1901). Some experimental and clinical observations concerning states of increased intracranial tension. The Mutter lecture for 1901. Am. J. Med. Sci. 124: 375-400.

Dahlstrom, A. and Fuxe, K. (1964). Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brainstem neurons. Acta Physiol. Scand. 232 (62): 1-55.

Dahlstrom, A. and Fuxe, K. (1965). Evidence for the existence of monoamine neurons in the central nervous system. II. Experimentally induced changes in the intra-neutronal levels of bulbospinal neuron systems. Acta Physiol. Scand. 247: 1-36.

Daly, J.W., Padgett, W., Nimitkitpaisan, Y., Creveling, C.R., Cantacuzene, D. and Kirk, K.L. (1980). Fluoronorepinephrine: Specific agonists for the activation of alpha and beta-adrenergic sensitive cyclic AMP-generating systems in brain slices. J. Pharmacol. Exp. Ther. 212: 382-389.

Daly, J.W., Padgett, W. and Seamon, K.B. (1982). Activation of cyclic AMP-generating systems in brain membranes and slices by the diterpene forskolin. Augmentation of receptor-mediated responses. J. Neurochem. 38: 532-544.

Dampney, R.A.L., Czachurski, J., Dembowsky, K., Goodchild, A.K. and Seller, H. (1987). Afferent connections and spinal projections of the pressor region in the rostral ventrolateral medulla of the cat. J. Auton. Nerv. Syst. 20: 73-86.

Dampney, R.A.L., Goodchild, A.K., Robertson, L.G. and Montgomery, W. (1982). Role of ventrolateral medulla in vasomotor regulation : a correlative anatomical and physiological study. Brain Res. 249: 223-235.

Dampney, R.A.L. and Moon, E.A. (1980). Role of ventrolateral medulla in vasomotor response to cerebral ischemia. Am. J. Physiol. 239: H349-H358.

Davenport, A.P., Nunez, D.J., Hall, J.A., Kaumann, A.J. and Brown, M.J. (1989). Autoradiographical localisation of binding sites for porcine [^{125}I] Endothelin-1 in humans, pigs and rats: Functional relevance in humans. J. Cardiovasc. Pharmacol. 73 (Suppl.5): S166-S170.

Day, T.A., Ro, A. and Renaud, L. (1983). Depressor area within caudal ventrolateral medulla of the rat does not correspond to the A_1 catecholamine cell group. Brain Res. 279: 299-302.

De Nucci, G., Thomas, R., D'Orleans-Juste, P., Antunes, E., Walder, C., Warner, T.D. and 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. Proc. Natl. Acad. Sci. 85: 9797-9800.

De Weid, D. and Jolles, J. (1982). Neuropeptides derived from pro-opicortin: behavioural, physiological and neurochemical effects. Physiol. Rev. 62: 976-1059.

Di Maggio, D.A., Chronwall, B.M., Buchanan, K. and O'Donohue, T.L. (1985). Pancreatic polypeptide immunoreactivity in rat brain is actually neuropeptide Y. Neurosci. 15: 1149-1157.

Dittmar, C. (1873). Uber die Hage des sogenannten Gefasscentrums in der Medulla Oblongata. Ber. Sachs. Akad. Wiss. 25: 18-45.

Doba, N. and Reis, D.J. (1973). Acute fulminating neurogenic hypertension produced by brainstem lesions in the rat. Circ. Res. 32: 584-593.

Duman, R.S. and Enna, S.J. (1986). A procedure for measuring α_2-adrenergic receptor-mediated inhibition of cAMP accumulation in rat brain slices. Brain Res. 384: 391-394.

Dunwiddie, T.V. (1985). The physiological role of adenosine in the central nervous system. Int. Rev. Neurobiol. 27: 63-139.

Eccles, J.C., Fatt, P. and Koketsu, K. (1954). Cholinergic and inhibitory synapses in a pathway from motor axon collaterals to motoneurones. J. Physiol. 126: 524-562.

Edvinsson, L. (1986). Characterisation of the contractile effect of neuropeptide Y in feline cerebral arteries. Acta Physiol. Scand. 125: 33-41.

Edvinsson, L., Copeland, J.R., Emson, P.C., McCulloch, J. and Uddman, R. (1987). Nerve fibres containing neuropeptide Y in the cerebrovascular bed: Immunocytochemistry, radioimmunoassay and vasomotor effects. J. Cereb. Blood Flow Metab. 7: 45-57.

Edvinsson, L., Ekblad, E., Hakanson, R. and Wahlestedt, C. (1984a). Neuropeptide Y potentiates the effect of various vasoconstrictor agents on rabbit blood vessels. Br. J. Pharmacol. 83: 519-525.

Edvinsson, L., Emson, P., McCulloch, J., Tatemoto, K. and Uddman, R. (1983). Neuropeptide Y: Cerebrovascular innervation and vasomotor effects in the cat. Neurosci. Lett. 43: 79-84.

Edvinsson, L., Emson, P., McCulloch, J., Tatemoto, K. and Uddman, R. (1984b). Neuropeptide Y: Immunocytochemical localisation to and effect upon feline pial arteries and veins in vitro and in situ. Acta. Physiol. Scand. 122: 155-163.

Edvinsson, L., Hakanson, R., Steen, S., Sundler, F., Uddman, R. and Wahlestedt, C. (1985). Innervation of human omental arteries and veins and vasomotor responses to noradrenaline, neuropeptide Y, substance P and vasoactive intestinal polypeptide. Regul. Pep. 12: 67-79.

Ekblad, E., Edvinsson, L., Wahlestedt, C., Uddman, R., Hakanson, R. and Sundler, F. (1984). Neuropeptide Y co-exists and co-operates with noradrenaline in perivascular nerve fibres. Regul. Pep. 8: 225-235.

Ernsberger, P.R., Meeley, M.P. and Reis, D.J. (1986). An endogenous clonidine-like substance binds preferentially to imidazole binding sites in the ventrolateral medulla labelled by ^{3}H-para-aminoclonidine. J. Hypertension 4 (Suppl.5): S109-S111.

Etgen, A.M. and Petitti, N. (1987). Mediation of norepinephrine-stimulated cyclic AMP accumulation by adrenergic receptors in hypothalamic and preoptic area slices: Effects of Estradiol. J. of Neurochem. 49: 1732-1739.

Everitt, B.J., Hokfelt, T., Terenius, K., Tatemoto, K., Mutt, V. and Goldstein, M. (1984). Differential coexistence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. Neurosci. 11 (2): 443-462.

Ferrario, C.M., Dickinson, C.J. and McCubbin, J.W. (1970). Central vasomotor stimulation by angiotensin. Clin. Sci. 39: 239-245.

Flint Beal, M., Frank, R.C., Ellison, D.W. and Martin, J.B. (1986). The effect of neuropeptide Y on striatal catecholamines. Neurosci. Lett. 71: 118-123.

Franco-Cereceda, A., Lundberg, J.M. and Dahlof, C. (1985). Neuropeptide Y and sympathetic control of heart contractility and coronary vascular tone. Acta Physiol. Scand. 124: 361-369.

Fredholm, B.B., Jansen, I. and Edvinsson, L. (1985). Neuropeptide Y is a potent inhibitor of cyclic AMP accumulation in feline cerebral blood vessels. Acta Physiol. Scand. 124: 467-469.

Fredholm, B.B., Jonzon, B. and Lindstrom (1986). Effect of adenosine receptor agonists and other compounds on cyclic AMP accumulation in forskolin-treated hippocampal slices. Naunyn-Schmiederberg's Arch. Pharmacol. 332: 173-178.

Fried, G., Lundberg, J.M. and Theodorsson-Norheim, E. (1985a). Subcellular storage and axonal transport of neuropeptide Y (NPY) in relation to catecholamines in the cat. Acta Physiol. Scand. 125: 145-154.

Fried, G., Terenius, L., Hokefet, T. and Goldstein, M. (1985b). Evidence for differential localisation of noradrenaline and neuropeptide Y (NPY) in neuronal storage vesicles isolated from rat vas deferens. J. Neurosci. 5: 450-458.

Fuxe, K., Agnati, L.F., Andersson, K. et al (1984). Receptor-receptor interactions in the central nervous system. Evidence for the existence of heterostatic synaptic mechanisms. In: Vizi, E.S. and Magyer, K. (eds.). Regulation of transmitter function: Basic and Clinical Aspects, Elsevier Science Publ.: 129-140.

Fuxe, K., Agnati, L.F., Harfstrand, A.et al (1986). Morphofunctional studies on the neuropeptide Y/adrenaline costoring terminal systems in the dorsal cardiovascular region of the medulla oblongata. Focus on receptor-receptor interactions in cotransmission. Prog. Brain Res. 68: 303-320.

Fuxe, K., Agnati, L.F., Harfstrand, A., Zini, I., Tatemoto, K., Pich, E.M., Hokfelt, T., Mutt, V. and Terenius, L. (1983). Central administration of neuropeptide Y induces hypotension, bradypnea and EEG synchronisation in the rat. Acta Physiol. Scand. 118: 189-192.

Fuxe, K., Harfstrand, A. and Agnati, L. (1987a). Neuropeptide Y - Adrenaline in central cardiovascular regulation. Neurosci. 22: S391.

Fuxe, K., Harfstrand, A., Agnati, L.F. et al (1987b). Central catecholamine - Neuropeptide Y interactions at the pre- and post-synaptic level in cardiovascular centers. J. Cardiovasc. Pharmacol. 10 (Suppl.12): S1-S13.

Fuxe, K., von Euler, G., van der Ploeg, I., Fredholm, B.B. and Agnati, L.F. (1989). Pertussin toxin treatment counteracts the cardiovascular effects of neuropeptide Y and clonidine in the awake unrestrained rat. Neurosci. Lett. 101: 337-341.

Ganten, D. and Pfaff, D. (1983). Central cardiovascular control. Basic and clinical aspects. In: Current topics in neuroendocrinology. Springer-Verlag, Heidelberg 13: 31.

Gardiner, S.M. and Bennett, T. (1989). Brain neuropeptides: actions on central cardiovascular control mechanisms. Brain Res. Rev. 14: 79-116.

Gardiner, S.M., Compton, A.M. and Bennett, T. (1989). Regional haemodynamic effects of Endothelin-1 in conscious, unrestrained, Wistar rats. J. Cardiov. Pharmacol. 13 (Suppl.5): S202-S204.

Gauthier, P., Reis, D.J. and Nathan, M.A. (1981). Arterial hypertension elicited either by lesions or by electrical stimulations of the rostral hypothalamus in the rat. Brain Res. 211: 91-105.

Gebber, G.L. (1984). Brainstem systems involved in cardiovascular regulation. In: Nervous control of cardiovascular function. Randall, W.C. (ed.). Oxford Univ. Press, New York: 346-368.

Gehlert, D.R., Dawson, T.M., Yamamura, H.I. and Wasley, J.K. (1985). Quantitative autoradiography of [^{3}H] forskolin binding sites in the rat brain. Brain 361: 351-360.

Geis, G.S., Kozelka, J.W. and Wurster, R.D. (1981). Organisation and reflex control of vagal cardiomotor neurons. J. Autonom. Nerv. Syst. 3: 437-442.

Geis, G.S. and Wurster, R.D. (1980). Cardiac responses during stimulation of the dorsal motor nucleus and nucleus ambiguus in the cat. Circ. Res. 46: 606-611.

Gibson, S.J., Polak, J.M., Allen, J.M., Adrian, T.E., Kelly, J.S. and Bloom, S.R. (1984). The distribution and origin of a novel brain peptide, neuropeptide Y, in the spinal cord of several mammals. J. Comp. Neurol. 227: 78-91.

Gillis, R.A., Helke, C.J., Hamilton B.L., Norman, W. and Jacobowitz, M. (1980). Evidence that substance P is a neurotransmitter of baro- and chemoreceptor afferents in nucleus tractus solitarius. Brain Res. 181: 476-481.

Glover, W.G. (1985). Increased sensitivity of rabbit ear artery to noradrenaline following perivascular nerve stimulation may be a response to neuropeptide Y release as a cotransmitter. Clin. Exp. Pharmacol. Physiol. 12: 227-231.

Goldstein, M., Kusano, N., Adler, C. and Meller, E. (1986). Characterisation of central neuropeptide Y receptor binding sites and possible interactions with α_2-adrenoceptors. Prog. Brain Res. 68: 331-335.

Gordon, F.J. (1987). Aortic baroreceptor reflexes are mediated by NMDA receptors in caudal ventrolateral medulla. Am. J. Physiol. 252: R628-R633.

Goto, K., Kasuya, Y., Matsuki, N. et al (1989). Endothelin activates the dihydropyridine-sensitive, voltage-dependent Ca^{2+} channel in vascular smooth muscle. Proc. Natl. Acad. Sci. 86: 3915-3918.

Grady, P.A. and Blaumanis, O.R. (1988). Physiologic parameters of the Cushing Reflex. Surg. Neurol. 29: 454-461.

Granata, A.R., Humao, Y., Kumada, M. and Reis, D.J. (1984). Rostral ventrolateral medulla area containing C_1 adrenaline neurones mediates sympathetic cardiovascular responses elicited from brainstem area containing A_1 noradrenergic neurons. Neurosci. Abstr. 10: 34.

Granata, A.R., Numao, Y., Kumada, M. and Reis, D.J. (1986). A_1 noradrenergic neurones tonically inhibit sympathoexcitatory neurones of C_1 area in rat brainstem. Brain Res. 377: 127-146.

Granata, A.R., Ruggiero, D.A., Park, D.H., Joh, T.H. and Reis, D.J. (1983). Lesions of the epinephrine neurones in the rostral ventrolateral medulla abolish the vasodepressor components of baroreflex and cardiopulmonary reflex. Hyperten. 5 (Suppl.V): V-80-V-84.

Granata, A.R., Ruggiero, D.A., Park, D.H., Joh, T.H. and Reis, D.J. (1985). Brainstem area with C_1 epinephrine neurons mediates baroreflex vasodepressor responses. Am. J. Physiol. 248: H547-H567.

Gray, T.S. and Morley, J.E. (1986). Neuropeptide Y: Anatomical distribution and possible function in mammalian nervous system. Life Sciences 38: 389-401.

Gu, J., Adrian, T.E., Tatemoto, K., Polak, J.M., Allen, J.M. and Bloom, S.R. (1983). Neuropeptide tyrosine (NPY) - A major cardiac neuropeptide. Lancet i: 1008-1010.

Guertzenstein, P.G. (1973). Blood pressure effects obtained by drugs applied to the ventral surface of the brainstem. J. Physiol. 229: 395-408.

Gurtu, S., Sinha, J.N. and Bhargava, K.P. (1982). Involvement of $alpha_2$-adrenoceptors of nucleus tractus solitarius in baro-reflex mediated bradycardia. Naunyn-Schmiedeberg's Arch. Pharmacol. 321: 38-43.

Guyenet, P.G. and Cabot, J.B. (1981). Inhibition of sympathetic preganglionic neurones by catecholamines and clonidine: mediation by an alpha-adrenergic receptor. J. Neurosci. 1: 908-917.

Haeusler, G. (1974). Clonidine induced inhibition of sympathetic nerve activity: no indication for a central presynaptic or indirect sympathomimetic-mode of action. Naunyn-Schmiedeberg's Arch. Pharmacol. 286: 97-111.

Haeusler, G. (1982). Central alpha-adrenoceptors involved in cardiovascular regulation. J. Cardiovasc. Pharmacol. 4: S72-S76.

Haeusler, G., Gerold, M. and Thoenen, T. (1972). Cardiovascular effects of 6-hydroxydopamine injected into a lateral brain ventricle of the rat. Naunyn-Schmiedeberg's Arch. Pharmacol. 272: 211-228.

Haggblad, J. and Fredholm, B.B. (1987). Adenosine and neuropeptide Y enhance α_1-adrenoceptor-induced accumulation of inositol phosphates and attenuate forskolin-induced accumulation of cAMP in rat vas deferens. Neurosci. Lett. 82: 211-216.

Halliday, G.M., Li, Y.W., Oliver, J.R., Joh, T.H., Cotton, R.G.H., Howe, P.R.C., Geffen, L.B. and Blessing, W.W. (1988). The distribution of neuropeptide Y-like immunoreactive neurons in the human medulla oblongata. Neurosci. 26 (1): 179-191.

Han, S.P., Trapani, A.J., Fok, K.F., Westfall, T.C. and Knuepfer, M.M. (1989). Effects of endothelin on regional haemodynamics in conscious rats. Eur. J. Pharmacol. 159: 303-305.

Harfstrand, A. (1986). Intraventricular administration of neuropeptide Y (NPY) induces hypotension, bradycardia and bradypnoea in the awake unrestrained male rat. Counteraction by NPY-induced feeding behaviour. Acta. Physiol. Scand. 128: 121-123.

Harfstrand, A., Eneroth, P., Agnati, L.F. and Fuxe, K. (1987b). Further studies on the effects of central administration of neuropeptide Y on neuroendocrine function in the male rat: Relationship to hypothalamic catecholamines. Regul. Peptides 17: 167-179.

Harfstrand, A., Fredholm, B. and Fuxe, K. (1987c). Inhibitory effects of neuropeptide Y on cyclic AMP accumulation in slices of the nucleus tractus solitarius region of the rat. Neurosci. Lett. 76: 185-190.

Harfstrand, A. and Fuxe, K. (1987). Simultaneous central administration of adrenaline and neuropeptide Y leads to antagonistic interactions in vasodepressor responses in awake male rats. Acta Physiol. Scand. 130: 529-531.

Harfstrand, A., Fuxe, K., Agnati, L.F., Benfenati, F. and Goldstein, M. (1986). Receptor autoradiographical evidence for high densities of ^{125}I-neuropeptide Y binding sites in the nucleus tractus solitarius of the normal male rat. Acta Physiol. Scand. 128: 195-200.

Harfstrand, A., Fuxe, K., Agnati, L. and Fredholm, B. (1989). Reciprocal interactions between α_2-adrenoceptor agonist and neuropeptide Y binding sites in the nucleus tractus solitarius of the rat. J. Neural. Transm. 75: 83-99.

Harfstrand, A., Fuxe, K., Agnata, L.F., Ganten, D., Eneroth, P., Tatemoto, K. and Mutt, V. (1984). Studies on neuropeptide Y catecholamine interactions in central cardiovascular regulation in the -chloralose anaesthetised rat. Evidence for a possible new way of activating the α_2-adrenergic transmission line. Clin. and Exper. - Theory and Practice A6 (10 and 11): 1947-1950.

Harfstrand, A., Fuxe, K., Terenius, L. and Kalia, M. (1987a). Neuropeptide Y-Immunoreactive perikarya and nerve terminals in the rat medulla oblongata: Relationship to cytoarchitecture and catecholamine cell groups. J. Comp. Neurol. 260: 20-35.

Harland, D., Bennett, T. and Gardiner, S.M. (1988). Cardiovascular actions of neuropeptide Y in the hypothalamic paraventricular nucleus of conscious Long Evans and Brattleboro rats. Neurosci. Lett. 85: 239-243.

Harper, A.M. and MacKenzie, E.T. (1977). Cerebral circulatory and metabolic effects of 5-hydroxytryptamine in anaesthetised baboons. J. Physiol. 271: 721-733.

Harris, M.C. (1979). Effects of chemoreceptor and baroreceptor stimulation on the discharge of hypothalamic supraoptic neurons in rats. J. Endocrinol. 82: 115-125.

Hassal, C.J.S. and Burnstock, G. (1989). Neuropeptide Y-like immunoreactivity in cultured intrinsic neurones of the heart. Neurosci. Lett. 52: 111-115.

Head, G.A., Badoer, E. and Korner, P.I. (1987b). Cardiovascular role of A_1 catecholaminergic neurons in the rabbit. Effect of chronic lesions on responses to methyldopa, clonidine and 6-OHDA induced transmitter release. Brain Res. 412: 18-28.

Head, G.A., Quail, A.W. and Woods, R.L. (1987a). Lesions of the A_1 noradrenergic cells affect AVP release and heart rate during haemorrhage. Am. J. Physiol. 253: H1012-1017.

Hendry, S.H.C., Jones, E.G., Defelipe, J., Schmechel, D., Brandon, C. and Emson, P.C. (1984). Neuropeptide-containing neurons of the cerebral cortex are also GABAergic. Proc. Natl. Acad. Sci. 81: 6526-6530.

Herkenham, M. (1987). Mismatches between neurotransmitter and receptor localisations in brain. Observations and implications. Neurosci. 23 (1): 1-38.

Higuchi, H. (1989). Neuropeptode Y : Functions and biosynthesis as a peptidergic neurotransmitter and the regulation of neuron-specific expression of NPY gene. Folia Pharmacol. Japon 93: 203-218.

Hilton, S.M., Marshall, J.M. and Timms, R.J. (1983). Ventral medullary relay neurones in the pathway from the defence areas of the cat and their effect on blood pressure. J. Physiol. 345: 149-166.

Hinson, J., Rauh, C. and Coupet, J. (1988). Neuropeptide Y stimulates phospholipid hydrolysis in rat brain miniprisms. Brain Res. 446 (2): 379-382.

Hirata, Y., Yoshimi, H., Takata, S., Watanabe, T.Y., Kumagai, S., Nakajima, K. and Sakakibara, S. (1988). Cellular mechanism of action by a novel vasoconstrictor endothelin in cultured rat vascular smooth muscle cells. Biochem. Biophys. Res. Commun. 154 (No.3): 868-875.

Hokfelt, T., Everitt, B.J., Theodorsson-Norheim, E. and Goldstein, M. (1984b). Occurrence of neurotensin-like immunoreactivity in subpopulations of hypothalamic, mesencephalic and medullary catecholamine neurons. J. Comp. Neurol. 222: 543-559.

Hokfelt, T., Fuxe, K., Goldstein, M. and Johansson, O. (1974). Immunohistochemical evidence for the existence of adrenaline neurones in the rat brain. Brain Res. 66: 235-251.

Hokfelt, T., Holets, V.R., Staines, W. et al (1986). Coexistence of neuronal messengers - an overview. Prog. Brain Res. 68: 33-70.

Hokfelt, T., Johansson, O. and Goldstein, M. (1984a). Chemical anatomy in the brain. Science 225: 1326-1334.

Hokfelt, T., Lundberg, J.M., Tatemoto, K. et al (1983a). Neuropeptide Y (NPY)-and FMRF-amide neuropeptide-like immunoreactivities in catecholamine neurons of the rat medulla oblongata. Acta Physiol. Scand. 117, 315-318.

Hokfelt, T., Skirball, L., Everitt, B.J. et al (1985). Distribution of choleoystokinin-like immunoreactivity in the nervous system with special reference to coexistence with classical neurotransmitters and other neuropeptides. In: Vanderhaeghen, J.J. and Crawley, J. (eds.). Neuronal Cholecystokinin. Ann. N.Y. Acad. Sci.

Howe, P.R.C. (1985). Blood pressure control by neurotransmitters in the medulla oblongata and spinal cord. J. Auton. Nerv. Syst. 12: 95-115.

Howe, P.R.C., Costa, J.B., Furness, J.P. and Chalmers, J.P. (1980). Simultaneous demonstration of phenylethanolamine N-methyltransferase immunofluorescent and catecholamine fluorescent nerve cell bodies in the rat medulla oblongata. Neurosci. 5: 2229-2238.

Hoyer, D. Waeber, C. and Palacios, J.M. (1989). [^{125}I] Endothelin-1 binding sites: Autoradiographic studies in the brain and periphery of various species including human. J. Cardiov. Pharmacol. 13 (Suppl.5): S162-S165.

Hu, J.R., Berninger, U.G. and Lang, R.E. (1988). Endothelin stimulates atrial natriuretic peptide (ANP) release from rat atria. Eur. J. Pharmacol. 158: 177-178.

Illes, P. (1986). Mechanisms of receptor-mediated modulation of transmitter release in noradrenergic, cholinergic and sensory neurones. Neurosci. 17 (4): 909-928.

Inoue, A., Yanagisawa, M., Kimura, S., Kasuya, Y., Miyauchi, T., Goto, K. and Masaki, T. (1989). The human endothelin family: Three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc. Natl. Acad. Sci. 86: 2863-2867.

Ishikawa, T., Yanagisawa, M., Kimura, S., Goto, K. and Masaki, T. (1988a). Positive inotropic action of novel vasoconstrictor peptide endothelin on guinea pig atria. Am. J. Physiol. 255: H970-H973.

Ishikawa, T., Yanagisawa, M., Kimura, S., Goto, K. and Masaki, T. (1988b). Positive chronotropic effects of endothelin, a novel endothelium-derived vasoconstrictor peptide. Pflugers Arch. 413: 108-110.

Jakobs, K.H. (1985). Coupling mechanisms of $alpha_2$-adrenoceptors. J. Cardiovasc. Pharmacol. 7 (Suppl.6): 109-112.

Jessell, T.M., Emson, P.C., Paxinos, G. and Cuello, A.C. (1978). Topographic projections of substance P and GABA pathways in the striato- and pallido-nigral system: A biochemical and immunohistochemical study. Brain Res. 152: 487-498.

Johnston, C.I. (1985). Vasopressin in circulatory control and hypertension. J. Hyperten. 3: 557-569.

Jones, C.R., Hiley, C.R., Pelton, J.R. and Mohr, M. (1989). Autoradiographic visualisation of the binding sites for [^{125}I] endothelin in rat and human brain. Neurosci. Lett. 97: 276-279.

Joseph, S.K. (1985). Receptor-stimulated phosphoinositide metabolism: a role for GTP-binding proteins? Trends Biol. Sci. 10: 297-298.

Kalia, M. (1981). Brain stem location of vagal preganglionic neurons. J. Auton. Nerv. Syst. 3: 451-481.

Kassis, S., Olasmaa, M., Terenius, L. and Fishman, P.H. (1987). Neuropeptide Y inhibits cardiac adenylate cyclase through a pertussis toxin-sensitive G protein. J. Biol. Chem. 262 (8): 3429-3431.

Kasuya, Y., Takuwa, Y., Yanagisawa, M., Kimura, S., Goto, K. and Masaki, T. (1989). Endothelin-1 induces vasoconstriction through two functionally distinct pathways in porcine coronary artery: contribution of phosphoinoside turnover. Biochem. and Biophys. Res. Comm. 163 (3): 1049-1055.

Katada, T., Amano, T. and Ui, M. (1982). Modification by islet-activating protein of adenylate cyclase activity in C_6 glioma cells. J. Biol. Chem. 193: 265-275.

Katada, T., Olnuma, M. and Ui, M. (1986). Mechanisms for inhibition of the catalytic activity of adenylate cyclase by the guanine nucleotide-binding proteins serving as the substrate of the islet activating protein, pertussis toxin. J. Biol. Chem. 261: 5215-5221.

Katada, T. and Ui, M. (1982). Direct modification of the membrane adenylate cyclase system by islet-activating protein due to ADP-ribosylation of a membrane protein. Proc. Natl. Acad. Sci. 79: 3129-3135.

Kawano, Y., Yoshida, K., Yoshimi, H., Kuramochi, M., Omae, T. (1989). Effects of intracerebroventricular endothelin on blood pressure and renal excretory function in the rat. 12th Scientific Meeting of the International Society of Hypertension, Milan, Italy: 419.

Kelly, E. and Nahorski, S.R. (1986). Specific inhibition of dopamine D-1-mediated cyclic AMP formation by dopamine D-2, muscarinic cholinergic, and opiate receptor stimulation in rat striatal slices. J. Neurochem. 47: 1512-1516.

Kent, R.S., DeLean, A. and Lefkowitz, R.J. (1980). A quantitative analysis of beta-adrenergic receptor interactions: Resolution of high and low affinity states of the receptor by compute modelling of ligand binding data. Mol. Pharmacol. 17: 14-23.

Kety, S.S. (1951). The theory and applications of the exchange of inert gas at the lungs and tissues. Pharmacol. Rev. 3: 1-41.

Kitamura, Y.Y., Nomura, Y. and Segawa, T. (1985). Possible involvement of inhibitory GTP binding regulatory protein in α_2-adrenoceptor mediated inhibition of adenylate cyclase activity in cerebral cortical membranes of rats. J. Neurochem. 45: 1504-1508.

Kitamura, Y., Nomura, Y. and Segawa, T. (1986). α_2-adrenoceptor-GTP binding regulatory protein adenylate cyclase system in cerebral cortical membranes of adult and senescent rats. Brain Res. 379: 118-124.

Kloog, Y. and Sokolowsky (1989). Simularities in mode and sites of action of safarotoxins and endothelins. Trends Pharmac. Sci. 10: 212-214.

Kobinger, W. (1978). Central -adrenergic systems as targets for hypotensive drugs. Rev. Physiol. Biochem. Pharmacol. 81: 39-100.

Koizumi, K. and Yamashita, H. (1978). Influence of atrial stretch receptors on hypothalamic neurosecretory neurons. J. Physiol. 285: 341-358.

Korner, P.I. and Head, G.A. (1983). Cardiovascular functions of central noradrenergic and serotinergic neurones in conscious rabbits and their contributions to the central actions of clonidine. Chest 83: 335-338.

Koseki, C., Imai, M., Hirata, Y., Yanagisawa, M. and Masaki, T. (1989a). Autoradiographic distribution in rat tissues of binding sites for endothelin: a neuropeptide? Am. J. Physiol. 256: R858-R866.

Koseki, C., Imai, M., Hirata, Y., Yanagisawa, M. and Masaki, T. (1989b). Binding sites for endothelin-1 in rat tissues: An autoradiographic study. J. Cardiov. Pharmacol. 13 (Suppl.5): S153-S154.

Kowall, N.W., Ferrante, R.J., Beal, M.F. and Martin, J.B. (1985). Characteristics, distribution and interrelationships of somatostatin, neuropeptide Y and NADPH-diaphorase in human caudal nucleus. Soc. Neurosci. Abst. 11: 209.

Kubo, T. and Kihara, M. (1987). Blood pressure modulation by substance P in the rat nucleus tractus solitarius. Brain Res. 413: 379-383.

Kubu, T. and Misu, Y. (1981). Pharmacological characterisation of the alpha-adrenoceptors responsible for a decrease of blood pressure in the nucleus tractus solitarii of the rat. Naunyn-Schmiedeberg's 317: 120-125.

Kurihara, H., Yoshizumi, M., Sugiyama, T. et al (1989). The possible role of endothelin-1 in the pathogenesis of coronary vasospasm. J. Cardiovasc. Pharmacol. 13 (Suppl.5): S132-S137.

Larhammar, D., Ericsson, A. and Persson, H. (1987). Structure and expression of the rat neuropeptide Y gene. Proc. Natl. Acad. Sci. 84: 2068-2072.

Lemmer, B.P., Lang, P-H., Schmidt, S. and Barmeier, H. (1987). Evidence for circadian rhythmicity of the β-adrenoceptor-adenylate cyclase-cAMP-phosphodiesterase system in the rat. J. Cardiovasc. Pharmacol. 10: S138-S143.

Leslie, R.A. (1985). Neuroactive substances in the dorsal vagal complex of the medulla oblongata: Nucleus of the solitary tract, area postrema, the dorsal motor nucleus of the vagus. Neurochem. Int. 7: 191-121.

Levitzki, A. (1987). Regulation of hormone-sensitive adenylate cyclase. Trends. Pharmacol. Sci. 8: 299-303.

Loewy, A.D. and Burton, H. (1978). Nuclei of the solitary tract: efferent projections to the lower brainstem and spinal cord of the cat. J. Comp. Neurol. 181: 421-450.

Lorenz, R.G., Saper, C.B., Wong, D.L., Ciaranello, R.D. and Loewy, A.D. (1985). Colocalisation of substance P - and phenylethanolamine-N-methyltransferase-like immunoreactivity in neurons of the ventrolateral medulla that project to the spinal cord: potential role in control of vasomotor tone. Neurosci. Lett. 55: 255-260.

Lowry, O.H., Roxbrough, N.J., Farr, A.L. and Randal, F.R.J. (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265-275.

Lundberg, J.M., Hemsen, A., Larsson, O., Rudehill, A., Saria, A. and Fredholm, B.B. (1988). Neuropeptide Y receptor in pig spleen: binding characteristics, reduction of cyclic AMP formation and calcium antagonist inhibition of vasoconstriction. Eur. J. Pharmacol. 145: 21-29.

Lundberg, J.M. and Hokfelt, T. (1983). Coexistence of peptides and classical neurotransmitters. Trends Neurosci. 6: 325-333.

Lundberg, J.M., Pernow, J., Tatemoto, K. and Dahlof, C. (1985). Pre- and post-junctional effects of NPY on sympathetic control of rat femoral artery. Acta. Physiol. Scand. 123: 511-513.

Lundberg, J.M., Rudehill, A., Sollevi, A., Theodrosson-Norheim, E., Pernow, J., Hamberger, B. and Goldstein, M. (1986). Frequency and reserpine-dependent chemical coding of sympathetic transmission: Differential release of noradrenaline and neuropeptide Y from pig spleen. Neurosci. Lett. 63: 96-100.

Lundberg, J.M. and Tatemoto, K. (1982). Pancreatic polypeptide family (APP, BPP, NPY and PYY) in relation to sympathetic vasoconstriction resistant to alpha-adrenoceptor blockade. Acta. Physiol. Scand. 116: 393-402.

Lundberg, J.M., Terenius, L., Hokfelt, T. and Goldstein, M. (1983). High levels of neuropeptide Y in peripheral noradrenergic neurons in various mammals including man. Neurosci. Lett. 42: 167-172.

Lundberg, J.M., Terenius, L., Hokfelt, T. and Tatemoto, K. (1984). Comparative immunohistochemical and biochemical analysis of pancreatic polypeptide-like peptides with special reference to the presence of neuropeptide Y in central and peripheral neurones. J. Neurosci. 4: 2376-2386.

Maccarrone, C. and Jarrett, B. (1985). Differences in regional brain concentrations of neuropeptide Y in spontaneously hypertensive (SH) and Wistar-Kyoto (WKY) rats. Brain Res. 345: 165-169.

Machado, B.H. and Brody, M.J. (1988a). Role of the nucleus ambiguus in the regulation of heart rate and arterial pressure. Hypertension 11: 602-607.

Machado, B.H. and Brody, M.J. (1988b). Effect of nucleus ambiguus lesion on the development of neurogenic hypertension. Hypertension 11 (Suppl.I): I-135-I-138.

Macrae, I.M., Dewar, D., Reid, J.L. and McCulloch, J. (1990). Specific alterations in cardiovascular function and in glucose utilisation within lower brainstem nuclei following intracisternal neuropeptide Y in the conscious rat. Brain Res. (in press).

Macrae, I.M. and Reid, J.L. (1988). Cardiovascular significance of neuropeptide Y in the caudal ventrolateral medulla of the rat. Brain Res. 456: 1-8.

Maley, B. and Elde, R. (1982). Immunohistochemical localisation of putative neurotransmitters within the feline nucleus tractus solitarii. Neurosci. 7: 2469-2490.

Marsden, P.A., Danlhuluri, N.R., Brenner, B.M., Ballermann, B.J. and Brock, T.A. (1989). Endothelin action on vascular smooth muscle involves inositol trisphosphate and calcium mobilisation. Biochem. Biophys. Res. Commun. 158 (No.1): 86-93.

Martel, J.C., St-Pierre, S. and Quirion, R. (1986). Neuropeptide Y receptors in rat brain: Autoradiographic localisation. Peptide 7: 55-60.

Martin, J.R., Beinfeld, M.C. and Westfall, T.C. (1988). Blood pressure increases after injection of neuropeptide Y into posterior hypothalamic nucleus. Am. J. Physiol. 254: H879-H888.

Martire, M., Fuxe, K., Pistritto, G., Preziosi, P. and Agnata, L.F. (1986). Neuropeptide Y enhances the inhibitory effects of clonidine on ^{3}H-noradrenaline release in synaptosomes isolated from the medulla oblongata of the male rat. J. Neural. Transmission 67: 113-124.

Medgett, J.C., McCulloch, M.H. and Rand, M.J. (1978). Partial agonist action of clonidine on prejunctional and postjunctional -adrenoceptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 304: 215-221.

Meeley, M.P., Ernsberger, P.R., Granata, A.R. and Reis, D.J. (1986). An endogenous clonidine-displacing substance from bovine brain: Receptor binding and hypotensive actions in the ventrolateral medulla. Life Sciences 38: 1119-1126.

Meeley, M.P., Ruggiero, D.A., Ishitsuka, T. and Reis, D.J. (1985). Intrinsic GABA neurones in the nucleus tractus solitarii and the rostral ventrolateral medulla of the rat: an immunohistochemical and biochemical study. Neurosi. Lett. 58: 83-89.

Melander, T., Hokfelt, T. and Rokaeus, A. (1986). Distribution of galanin-like immunoreactivity in the rat central nervous system. J. Comp. Neurol. 248: 475-517.

Milner, T.A., Picket, V.M., Park, D.H., Joh, T.H. and Reis, D.J. (1987). Phenylethanolamine N-methyltransferase-containing neurons in the rostral ventrolateral medulla of the rat. I. Normal ultrastructure. Brain Res. 411: 28-45.

Minson, J.B. and Chalmers (1984). Lesions of the ventrolateral medulla in normal and vasopressin-deficient (Brattleboro) rats. Clin. and Exp. Pharmacol. and Physiol. 11: 427-430.

Minth, C.D., Bloom, S.R., Polak, J.M. and Dixon, J.E. (1984). Cloning, characterisation and DNA sequence of a human cDNA encoding neuropeptide tyrosine. Proc. Natl. Acad. Sci. 81: 4577-4581.

Misu, Y., Fugie, K. and Kubo, T. (1982). Presynaptic dual inhibitory actions of guanabenz on adrenergic transmission. Eur. J. Pharmacol.: 77-81.

Mitchell, R. and Fleetwood-Walker, S. (1981). Substance P, but not TRH, modulates the 5-HT autoreceptor in ventral lumbar spinal cord. Eur. J. Pharmacol. 76: 119-120.

Mura, M. and Reis, D.J. (1971). The paramedian reticular nucleus: A site of inhibitory interaction between projections from fastigial nucleus and carotid sinus nerve acting on blood pressure. J. Physiol. 216: 441-466.

Moser, P.C. and Pelton, J.T. (1988). Behavioural effects of centrally administered endothelin in the rat. Proc. Br. Pharmacol. Soc.: 249P.

Motulsky, H.J. and Michel, M.C. (1988). Neuropeptide Y mobilises Ca^{2+} and inhibits adenylate cyclase in human erythroleukemia cells. Am. J. Physiol. 255: E880-E885.

Mraovitch, S., Kumada, M. and Reis, D.J. (1982). Role of the nucleus parabrachialis in cardiovascular regulation in cat. Brain Res. 232: 57-75.

McCulloch, J. (1988). The physiology and regulation of cerebral blood flow. In: Knezevic, S., Maximilian, V.A., Mubrin, Z., Prohovnik, I. and Wade, J. (eds). Handbook of Regional Cerebral Blood Flow. Lawrence Erlbaum Associates: 1-25.

McCulloch, J. and Edvinsson, L. (1980). Cerebral circulatory and metabolic effects of vasoactive intestinal polypeptide. Am. J. Physiol. 238: H449-H456.

McGillicuddy, J.E., Kindt, G.W., Raisis, J.E. and Miller, C.A. (1978). The relation of cerebral ischemia, hypoxia and hypercarbia to the Cushing Response. J. Neurosurg. 48: 730-740.

McLachlan, E.M. and Llewellyn-Smith, I.J. (1986). The immunohistochemical distribution of neuropeptide Y in lumbar pre- and paravertebral sympathetic ganglia of the guinea pig. J. Auton. Nerv. Sys. 17: 313-324.

Nakajima, T., Yashima, Y. and Nakamura, K. (1986). Quantitative autoradiographical localisation of neuropeptide Y receptors in the rat lower brainstem. Brain 380: 144-150.

Nakamichi, H., Murakami, M., Mizusawa, S. et al (1987). Effects of clonidine on the levels of cyclic nucleotides in the rat brain regions: The change of cyclic nucleotides after clonidine alone or in combination with antagonists. Folia Pharmacol. Japon 89: 331-337.

Nakamura, K., Gerold, M. and Thoenen, H. (1971). Experimental hypertension of the rat: Reciprocal changes of norepinophrine turnover in heart and brainstem. Naunyn-Schmiedeberg's Arch. Pharmacol. 268: 125-139.

Nield, T.O. (1987). Actions of neuropeptide Y on innervated and denervated rat tail arteries. J. Physiol. 386: 19-30.

Nelson, C.A. and Seamon, K.B. (1988). Binding of [^{3}H] forskolin to solubilised preparations of adenylate cyclase. Life Sci. 42 (No.14): 1375-1383.

Nichizuka, Y. (1988). The molecular heterogeneity of protein kinase C and its implications for cellular regulation. Nature 334: 616-664.

Nomura, Y., Kawata, K., Kitamura, Y. and Watanabe, H. (1987). Effects of pertussis toxin on the α_2-adrenoceptor-inhibitory GTP-binding protein-adenylate cyclase system in rat brain: Pharmacological and neurochemical studies. Eur. J. Pharmacol. 134: 123-129.

Norgren, R. (1978). Projections from the nucleus of the solitary tract in the rat. Neurosci. 3: 207-218.

Olasmaa, M., Pahlman, S. and Terenius, L. (1987). Beta-adrenoceptor, vasoactive intestinal polypeptide (VIP) and neuropeptide tyrosine (NPY) receptors functionally coupled to adenylate cyclase in the human neuroblastoma SK-N-MC cell line. Neurosci. Lett. 83: 161-166.

Olasmaa, M. and Terenius, R. (1986). Neuropeptide Y receptor interaction with β-adrenoceptor coupling to adenylate cyclase. Prog. Brain Res. 68: 337-341.

Owsjannikow, Ph. (1871). Die tonischen und reflectorischen zentra der gefassnerven. Ber. Sachs. Akad. Wiss. 23: 135-147.

Palkovits, M. and Brownstein, M.J. (1989). Catecholamines in the central nervous system. In: Trendelenburg, U. and Weiner, N. (eds.). Handbook of Experimental Pharmacology Vol.90 Part 2. Springer-Verlag: 1-22.

Paxinos, G. and Watson, P. (1986). The rat brain in stereotoxic coordinates. 2nd Edition, Academic Press Inc. (London).

Peres-Polon, V.L. and Correa, F.M.A. (1987). Involvement of central -pressor and -depressor adrenoceptors in the cardiovascular response to intracerebroventricular catecholamines in the rat. Gen. Pharmac. 18 (2): 159-164.

Perney, T.M. and Miller, R.J. (1989). Two different G-proteins mediate neuropeptide Y and bradykinin-stimulated phospholipid breakdown in cultured rat sensory neurones. J. Biol. Chem. 264 (13): 7317-7327.

Pernow, J., Lundberg, J.M. and Kayser, L. (1987). Vasoconstrictor effects in vivo and plasma disappearance rate of neuropeptide Y in man. Life Sci. 40: 47-54.

Petrenko, S., Olianas, M.C., Onali, P. and Gessa, G.L. (1987). Neuropeptide Y inhibits forskolin-stimulated adenylate cyclase activity in rat hippocampus. Eur. J. Pharmacol. 136: 425-428.

Petty, M.A., Dietrich, R. and Lang, R.E. (1984). The cardiovascular effects of neuropeptide Y (NPY). Clin. Exp. Hypertens. Theory Pract. A6 (10-11): 1889-1892.

Petty, M.A. and Reid. (1981). Opiate analogs, substance P and baroreceptor reflexes in the rabbit. Hypertension 3 (Suppl.I): I-142-I-147.

Peuler, J.D. and Johnson, G.A. (1977). Simultaneous single isotope radioenzymatic assay of plasma norepinephrine, epinephrine and dopamine. Life Sci. 21: 625-636.

Pilc, A. and Enna, S.J. (1985). Synergistic interaction between α- and β-adrenoceptors in rat brain cortical slices: Possible site for antidepressant drug action. Life Sci. 37: 1183-1194.

Pilc, A. and Enna, S.J. (1986). Activation of α_2-adrenergic receptors augments neurotransmitter-stimulated cyclic AMP accumulation in rat brain cerebral cortical slices. J. Pharmacol. Exp. Therap. 237: 725-730.

Pilowsky, P.M., Morris, M.J., Minson, J.B., West, M.J., Chalmers, J.P., Willoughby, J.O. and Blessing, W.W. (1987). Inhibition of vasodepressor neurons in the caudal ventrolateral medulla of the rabbit increases both arterial pressure and the release of neuropeptide Y-like immunoreactivity from the spinal cord. Brain Res. 420: 380-384.

Planitz (1987). Comparison of moxonidine and clonidine HCl in treating patients with hypertension. J. Clin. Pharmacol. 27: 46-51.

Porter, J.P., Bonham, C., Mangiapane, M.L., Webb, R.L., Brody, M.J. (1985). The cardiovascular effects of centrally and peripherally administered indoramin in conscious rats. Eur. J. Pharmacol. 109: 9-17.

Punnen, S., Urbanski, R., Krieger, A.J. and Sapru, H.N. (1987). Ventrolateral medullary pressor area: site of hypotensive action of clonidine. Brain Res. 442: 336-346.

Quirion, R., Gaudreau, P., St-Pierre, S., Rioux, F. and Pert, C.B. (1982). Autoradiographic distribution of [^{3}H]-neurotensin receptors in the rat brain: visualisation by tritum-sensitive film. Peptides 3: 757-763.

Regoli, D., Park, W.K. and Rioux, F. (1974). Pharmacology of angiotensin. Pharmac. Rev. 26: 69-123.

Reid, J.L.R. and Rubin, P.C. (1987). Peptides and central neural regulation of the circulation. Physiol. Rev. 67 (3): 725-749.

Reid, J.L. and Rubin, P.C. (1989). Catecholamines and blood pressure. In: Trendelenburg, U. and Weiner, N. (eds.). Handbook of Experimental Pharmacology 90 (Part 2), Springer-Verlag: 319-344.

Reid, J.L. Zivin, J.A. and Kopin, I.J. (1976). The effects of spinal cord transection and intracisternal 6-hydroxydopamine on phenylethanolamine-N-methyl transferase (PNMT) activity in rat brain stem and spinal cord. J. Neurochem. 26: 629-631.

Reis, D.J. (1987). Neurotransmitters acting in the C_1 area on the tonic and reflex control of blood pressure. J. of Cardiovasc. Pharmacol. 10 (Suppl.12): S22-S25.

Reis, D.J., Morrison, S. and Ruggiero, D.A. (1988). The C_1 area of the brainstem in tonic and reflex control of blood pressure. Hypertension II (Suppl.I): I-8-I-13.

Reis, D.J., Perrone, M.H. and Talman, W.T. (1981). Evidence that glutamic acid is the neurotransmitter of baroreceptor afferents terminating in the nucleus tractus solitarius (NTS). J. Auton. Nerv. Syst. 3: 321-334.

Robberectit, P., Waelbrock, M., Chatelain, P., Camus, J.C. and Christophe, J. (1983). Inhibition of forskolin-stimulated cardiac adenylate cyclase activity by short-chain alcohols. FEBS Lett. 154: 205-209.

Rockhold, R.W. and Caldwell, R.W. (1979). Effect of lesions of the nucleus tractus solitarii on the cardiovascular actions of clonidine in conscious rats. Neuropharmacol. 18: 347-354.

Rockhold, R.W. and Caldwell, R.W. (1980). Cardiovascular effects following clonidine microinjection into the nucleus tractus solitarii of the rat. Neuropharmacol. 19: 919-922.

Rodbell, M. (1980). The role of hormone receptors and GTP-regulatory proteins in membrane transduction. Nature 248: 17-22.

Ross, C.A., Armstrong, D.M., Ruggiero, D.A., Pickel, V.M., Joh, T.H. and Reis, D.J. (1981a). Adrenaline neurones in the rostral ventrolateral medulla innervate thoracic spinal cord: a combined immunocytochemical and retrograde transport demonstration. Neurosci. 25: 257-262.

Ross, C.A., Ruggiero, D.A., Joh, T.H., Park, D.H. and Reis, D.J. (1984a). Rostral ventrolateral medulla: selective projections to the thoracic autonomic cell column from the region containing C_1 adrenaline neurones. J. Comp. Neurol. 228: 168-184.

Ross, C.A., Ruggiero, D.A., Park, D.H., Toh, T.H., Sved, A.F., Fernandez-Pardal, J., Saavedra, J.M. and Reis, D.J. (1984b). Tonic vasomotor control by the rostral ventrolateral medulla: Effect of electrical or chemical stimulation of the area containing C_1 adrenaline neurones on arterial pressure, heart rate and plasma catecholamines and vasopressin. J. Neurosci. 4: 474-494.

Ross, C.A., Ruggiero, D.A. and Reis, D.J. (1981b). Afferent projections to cardiovascular portions of the nucleus of the tractus solitarius in the rat. Brain Res. 223: 402-408.

Ruggiero, D.A., Meeley, M.P., Anwar, M. and Reis, D.J. (1985a). Newly identified GABAergic neurons in regions of the ventrolateral medulla which regulate blood pressure. Brain Res. 339: 171-177.

Ruggiero, D.A., Ross, C.A., Anwar, M., Park, D.H., Joh, T.H. and Reis, D.J. (1985b). Distribution of neurons containing phenylethanolamine N-methyltransferase in the medulla and hypothalamus of rat. J. Comp. Neurol. 239: 127-154.

Saavedra, J.M., Grobecker, H., Axelrod, J. (1976). Adrenaline-forming enzyme in brainstem: elevation in genetic and experimental hypertension. Sci. 191: 483.

Saito, A., Shiba, R., Kimura, S., Yanagisawa, M., Goto, K. and Masaki, T. (1989). Vasoconstrictor response of large cerebral arteries of cats to endothelin, an endothelium-derived vasoactive peptide. Eur. J. of Pharmacol. 162: 353-358.

Sakurada, O., Kennedy, C., Jehle, J., Brown, J.D., Carbin, G.L. and Sokoloff, L. (1978). Measurement of local cerebral blood flow with iodo [^{14}C] antipyrine. Am. J. Physiol. 234 (1): H59-H66.

Saria, A., Theodorsson-Norheim, E. and Lundberg, J.M. (1984). Evidence for specific neuropeptide Y-binding sites in rat brain synaptosomes. Eur. J. Pharmacol. 107: 105-107.

Sawchenko, P.E. and Swanson (1982). The organisation of noradrenergic pathways from the brainstem to the paraventricular and supraoptic nuclei in the rat. Brain Res. Rev. 4: 275-325.

Sawchenko, P.E., Swanson, L.W., Grzanna, R., Howe, P.R.C., Bloom, S.R. and Polack, J.M. (1985). Colocalisation of neuropeptide Y immunoreactivity in brainstem catecholaminergic neurons that project to the paraventricular nucleus of the hypothalamus. J. Comp. Neurol. 241: 138-153.

Scatton, B., Pelayo, F., Dubocovich, M.L., Langer, S.Z. and Bartholini, G. (1979). Effects of clonidine on utilisation and potassium-evoked release of adrenaline in rat brain areas. Brain Res. 176: 197-201.

Schaz, K., Stock, G., Simon, W., Schlor, K.H. and Unger, T. (1980). Enkephalin effects on blood pressure, heart rate and baroreceptor reflex. Hyperten. 2: 395-407.

Schmitt, F.O. (1984). Molecular regulators of brain function: A new view. Neurosci. 13: 99-100.

Schmitt, H. and Schmitt, H. (1969). Localisation of the hypotensive effect of 2-(2,6-dichlorophenylamino)-2-imidazoline hydrochloride (ST 155, Catapresan). Eur. J. Pharmacol. 6: 8-12.

Schmitt, H., Schmitt, H., Boissier, J.R. and Guidicelli, J.F. (1967). Centrally mediated decrease in sympathetic tone induced by 2-(2,6-dichlorophenylamino)-2-imidazoline (ST 155, Catapresan). Eur. J. Pharmacol. 2: 147-148.

Seamon, K.B. and Daly, J.W. (1981). Activation of adenylate cyclase by the diterpene forskolin does not require the guanine nucleotide regulatory protein. J. Biol. Chem. 256: 9799-9801.

Seamon, K.B., Padgett, W. and Daly, J.W. (1981). Forskolin. Unique diterpene activator of adenylate cyclase in membranes and in intact cells. Proc. Natl. Acad. Sci. 78: 3363-3367.

Seller and Illert (1969). The localisation of the first synapse in the carotid sinus baroreceptor reflex pathway and its alteration of the afferent input. Pflugers Arch. 306: 1-19.

Shade, R.E. and Share, L. (1975). Vasopressin release during haemorrhage and angiotensin II infusion. Am. J. Physiol. 228: 149-154.

Shapiro, H.M., Greenberg, J.H., Reivich, M., Ashmead, G. and Sokoloff, L. (1978). Local cerebral glucose uptake in awake and halothane anaesthetised primates. Anaesthesiol. 48: 97-103.

Shapiro, R.E. and Miselis, R.R. (1985). The central neural connections of the area postrema of the rat. The J. of Comp. Neurol. 234: 344-364.

Sheikh, S.P., Hakanson, R. and Schartz, T.W. (1989). Y_1 and Y_2 receptors for neuropeptide Y. FEBS Lett. 245: 209-214.

Shigeno, T., Mima, T., Takakura, K., Yanagisawa, M., Saito, A., Goto, K. and Masaki, T. (1989). Endothelin-1 acts in cerebral arteries from the adventitial but not the luminal side. J. Cardiovasc. Pharmacol. 13 (Suppl.5): S174-S176.

Spyer, K.M. (1981). Neural organisation and control of the baroreceptor reflex. Rev. Physiol. Biochem. Pharmacol. 88: 24-124.

Stanley, B.G., Daniel, D.R., Chin, A.S., Leibowitz, S.F. (1985). Paraventricular nucleus injections of peptide YY and neuropeptide Y preferentially enhance carbohydrate ingestion. Peptides 6: 1205-1211.

Stanley, B.G. and Leibowitz, S.F. (1984). Neuropeptide Y: Stimulation of feeding and drinking by injection into the paraventricular nucleus. Life Sci. 35: 2635-2642.

Starke, K. and Montel, H. (1973). Involvement of alpha-receptors in clonidine-induced inhibition of transmitter release from central monoamine neurones. Neuropharmacol. 12: 1073-1080.

Stuesse, S.L. (1982). Origins of cardiac vagal preganglionic fibers: A retrograde transport study. Brain Res. 236: 15-25.

Stuesse, S.L. and Fish, S.E. (1984a). Projections to the cardioinhibitory region of the nucleus ambiguus of rat. J. Comp. Neurol. 229: 271-278.

Stuesse, S.L. and Fish, S.E. (1984b). Pathways for cardioinhibition in rat medulla. Anat. Rec. 208: 175a.

Stjarne, L., Lundberg, J.M. and Astrand, P. (1986). Neuropeptide Y - A cotransmitter with noradrenaline and adenosine 5'-trisphosphate in the sympathetic nerves of the mouse vas deferens? A biochemical, physiological and electropharmacological study. Neurosci. 18: 151-166.

Stone, E.A. and U'Prichard, D.C. (1981). [^{3}H] Dihydroalprenolol binding in the rat brainstem. Eur. J. of Pharmacol. 75: 159-161.

Stornetta, R.L., Guyenet, P.G. and McCarty, R.C. (1987). Autonomic nervous system control of hear rate during baroreceptor anaesthetised rats. J. of the Auton. Nerv. Syst. 20: 121-127.

Struyker Boudier, H.A.J., Smeets, G.W.M., Brouwer, G.M., van Rossum, J.M. (1974). Hypothalamic alpha adrenergic receptors in cardiovascular regulation. Neuropharmacol. 13: 837-846.

Sun, M.K., Filtz, T. and Guyenet, P.G. (1986). Role of glutamate in baroreflexes. Soc. Neurosci. Abstr. 12: 580.

Sun, M.K. and Guyenet, P.G. (1986). Effect of clonidine and γ-aminobutyric acid on discharges of medulla-spinal sympathoexcitatory neurones in the rat. Brain Res. 368: 1-17.

Sundler, F., Moghimzadeh, E., Hakanson, R., Ekelund, M. and Emson, P. (1983). Nerve fibres in the gut and pancreas of the rat displaying neuropeptide Y immunoreactivity. Cell Tissue Res. 230: 487-493.

Sutherland, E.W. and Rall, T.W. (1960). The relation of adenosine-3',5'-phosphate and phosphorylase to the actions of catecholamines and other hormones. Pharmacol. Rev. 12: 265-299.

Swanson and Kupers (1980). The paraventricular nucleus of the hypothalamus: cytoarchitectonic subdivisions and organisation of projections to the pituitary, dorsal vagal complex and spinal cord as demonstrated by retrograde fluorescence double-labelling methods. J. Comp. Neurol. 194: 555-570.

Szentagothai, J. (1952). The general visceral efferent column of the brainstem. Acta Morph. Acad. Sci. 2: 313-328.

Tabuchi, Y., Nakamaru, M., Rakugi, H., Nagano, M., Mikami, H. and Ogihara, T. (1989). Endothelin inhibits presynaptic adrenergic neurotransmission in rat mesenteric artery. Biochem. Biophys. Res. Commun. 161: 803-808.

Tatemoto, K. (1982). Neuropeptide Y: complete amino acid sequence of the brain peptide. Proc. Natl. Acad. Sci. 79: 5485-5489.

Tatemoto, K., Carlquist, M. and Mutt, V. (1982). Neuropeptide Y - a novel brain peptide with structural similarities to peptide YY and pancreatic poplypeptide. Nature 296: 659-660.

Thomas, M.R. and Calaresu, F.R. (1979). Localisation and function of medullary sites mediating vagal bradycardia in the cat. Am. J. Physiol. 226: 1344-1349.

Thompson, R.K. and Malina, S. (1959). Dynamic axial brainstem distortion as a mechanism explaining the cardiorespiratory changes in increased intracranial pressure. J. Neurosurg. 16: 664-675.

Timmermans, P.B.M.W.M. and Van Zwieten, P.A. (1980). Post-synaptic α_1- and α_2-adrenoceptors in the circulatory system of the pithed rat: selective stimulation of the α_2-type by BHT 933. Eur. J. Pharmacol. 63: 199-202.

Tomobo, T., Miyauchi, T., Saito, A., Yanagisawa, M., Kimura, S., Goto, K. and Masaki, T. (1988). Effects of endothelin on the renal artery from spontaneously hypertensive and Wistar Kyoto rats. Eur. J. Pharmacol. 152: 373-374.

Tovey, K.C., Oldham, K.G., Whelan, J.A.M. (1974). A simple direct assay for cyclic AMP in plasma and other biological samples using an improved competitive protein binding technique. Clinica Chimica Acta 56: 221-234.

Tuor, U.I., Kelly, P.A.T., Edvinsson, L. and McCulloch, J. (1987). Neuropeptide Y: Importance in the cerebral circulation. In: Edvinsson, L. and McCulloch, J. (eds.). Peptidergic mechanisms in the cerebral circulation. Ellis Horwood, Chichester: 79-99.

Uddman, R., Ekblad, E., Edvinsson, L., Hakansson, R. and Sundler, F. (1985). Neuropeptide Y-like immunoreactivity in perivascular nerve fibres of the guinea pig. Regul. Pept. 10: 243-257.

Uhlen, S. and Wikberg, J.E.S. (1988). Inhibition of cyclic AMP production by α_2-adrenoceptor stimulation in the guinea-pig in the guinea-pig spinal cord slices. Pharmacol. Toxicol. 63: 178-182.

Unden, A. and Bartfai, T. (1984). Regulation of neuropeptide Y (NPY) binding by guanine nucleotides in the rat cerebral cortex. FEBS Lett. 177 (1): 125-128.

Unden, A., Tatemoto, K., Mutt, V. and Bartfai, T. (1984). Neuropeptide Y receptor in the rat brain. Eur. J. Biochem. 145: 525-530.

Unger, T.H., Rascher, W., Schuster, C., Pavlovitch, R., Schomig, A., Dietz, R., Ganten, D. (1981). Central blood pressure effects of substance P and angiotensin II. Role of the sympathetic nervous system. Eur. J. Pharmacol. 71: 33-42.

Unnerstall, J.R., Kopajtic, T.A. and Kuhar, M.H. (1984). Distribution of α_2-agonist binding sites in the rat and human central nervous system: analysis of some functional anatomic correlates of the pharmacologic effects of clonidine and related adrenergic agonists. Brain Res. Rev. 319: 69-101.

U'Prichard, D.C., Greenberg, D.A. and Snyder, S.H. (1977). Binding characteristics of a radioligand agonist and antagonist at central nervous system alpha-noradrenergic receptors. Mol. Pharmacol. 13: 454-473.

Urbanski, R.W. and Sapru, H.N. (1988). Putative neurotransmitters involved in medullary cardiovascular regulation. J. Auton. Nerv. Syst. 25: 181-193.

Vallejo, D.A., Carter, S.B. and Lightman, S.L. (1987). Neuropeptide Y alters monoamine turnover in the rat brain. Neurosi. Lett. 73: 155-160.

Vallejo, M. and Lighman, S.L. (1986a). Pressor effect of centrally administered neuropeptide Y in rats: Role of sympathetic nervous system and vasopressin. Life Sci. 38: 1859-1866.

Vallejo, M. and Lightman, S.L. (1986b). Neuropeptide Y alters the haemodynamic responses to noradrenaline in the locus coeruleus of rats. Neurosci. Lett. 26 (Suppl.): S237.

Van Renterghem, C., Vigne, P., Barhanin, J., Schmid-Alliana, A., Frelin, C. and Lazdunski, M. (1988). Molecular mechanisms of action of the vasoconstrictor peptide endothelin. Biochem. Biophys. Res. Commun. 157 (3): 977-985.

Vincent, S.R. and Johansson, O. (1983). Striatal neurons containing both somatostatin and avian pancreatic polypeptide (APP)-like immunoreactivities and NADPH-diaphorase activity: a light and electron microscopic study. J. Comp. Neurol. 217: 267-270.

Vlahakos, D., Gavras, I. and Gavra, H. (1985). α-adrenoceptor agonists applied in the area of the nucleus tractus solitarii in the rat: effect of anaesthesia on cardiovascular responses. Brain Res. 347: 372-375.

Vogt, M. (1954). The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. J. Physiol. 123: 451-481.

Wahlestedt, C., Edvinsson, L., Ekblad, E. and Hakanson, R. (1985). Neuropeptide Y potentiates noradrenaline-evoked vasoconstriction: Mode of action. J. Pharmacol. Exp. Therap. 34: 735-741.

Wahlestedt, C., Skagerberg, G., Ekman, R., Heilig, M., Sundler, F. and Hakanson, R. (1987). Neuropeptide Y (NPY) in the area of the hypothalamic paraventricular nucleus activates the pituitary-adrenocortical axis in the rat. Brain Res. 417: 33-38.

Wahlestedt, C., Yanaihara, N. and Hakanson, R. (1986). Evidence for different pre- and post-junctional receptors for neuropeptide Y and related peptides. Reg. Peptides 13: 307-318.

Walker, M.W., Ewald, D.A., Perney, T.M. and Miller, R.J. (1988). Neuropeptide Y modulates neurotransmitter release and Ca^{2+} currents in rat sensory neurones. J. Neurosci. 8 (7): 2438-2446.

Walker, M.W. and Miller, R.J. (1988). ^{125}I-Neuropeptide Y and ^{125}I-peptide YY bind to multiple receptor sites in rat brain. Mol. Pharmacol. 34: 779-792.

Wallach, J.H. and Loewy, A.D. (1980). Projections of the aortic nerve to the nucleus tractus solitarius in the rabbit. Brain Res. 188: 247-251.

Wang, S.C. and Ranson, S.W. (1939). Autonomic responses to electrical stimulation of the lower brainstem. J. Comp. Neurol. 71: 437-455.

Ward-Routledge, C., Marshall, P. and Marsden, C.A. (1988). Involvement of central α- and β-adrenoceptors in the pressor responses to electrical stimulation of the rostral ventrolateral medulla. Br. J. Pharmacol. 94: 609-619.

Warner, T.D., De Nucci, G. and Vane, J.R. (1989). Rat endothelin is a vasodilator in the isolated perfused mesentery of the rat. Eur. J. Pharmacol. 159: 325-326.

Westfall, T.C., Martin, J., Chen, X. et al (1988). Cardiovascular effects and modulation of noradrenergic neurotransmission following central and peripheral administration of neuropeptide Y. Synapse 2: 299-307.

Westlind-Danielsson, A., Andell, S., Abens, J. and Bartfai, T. (1988). Neuropeptide Y and peptide YY inhibit adenylate cyclase activity in the rat striatum. Acta. Physiol. Scand. 132: 425-430.

Westlind-Danielsson, A., Unden, A., Abens, J., Andell, S. and Bartfai, T. (1987). Neuropeptide Y receptors and the inhibition of adenylate cyclase in the human frontal and temporal cortex. Neurosci. Lett. 74: 237-242.

Wikberg, J.E.S. and Hajos, M. (1987). Spinal cord α_2-adrenoceptors may be located postsynaptically with respect to primary sensory neurons: destruction of primary C-afferents with neonatal capsaicin ^{3}H-clonidine binding sites in mice. Neurosci. Lett. 76: 63-68.

Wiklund, N.P., Ohlen, A. and Cederqvist, B. (1988). Inhibition of adrenergic neuroeffector transmission by endothelin in the guinea-pig femoral artery. Acta. Physiol. Scand. 134: 311-312.

Willette, R.N., Punnen, S., Krieger, A.J. and Sapru, H.N. (1984). Interdependence of rostral and caudal ventrolateral medullary areas in the control of blood pressure. Brain Res. 321: 169-174.

Willette, R.N., Punnen-Grandy, S., Krieger, A.J. and Sapru, H.N. (1987). Differential regulation of regional vascular resistance by the rostral and caudal ventrolateral medulla in the cat. J. of Auton. Nerv. Syst. 18: 143-151.

Willoughby, J.O. and Blessing, W.W. (1987). Neuropeptide Y injected into the supraoptic nucleus causes secretion of vasopressin in the unanaesthetised rat. Neurosci. Lett. 75: 17-22.

Wing, L.M.H. and Chalmers, J.P. (1974). Effects of p-chlorophenylalanine on blood pressure and heart rate in normal rabbits and rabbits with neurogenic hypertension. Clin. Exp. Pharmacol. Physiol. 1: 219-229.

Winquist, R.J., Bunting, P.B., Garsky, V.M., Lumma, P.K. and Schofield, T.L. (1989). Prominent depressor response to endothelin in spontaneously hypertensive rats. Eur. J. Pharmacol. 163: 199-203.

Worley, P., Baraban, J.M., De Souza, E.B. and Snyder, S.H. (1986). Mapping second messenger systems in the brain: Differential localisations of adenylate cyclase and protein kinase C. Proc. Natl. Acad. Sci. 83: 4053-4057.

Wright, C.E. and Fozard, J.R. (1988). Regional vasodilation is a prominent feature of the haemodynamic response to endothelin in anaesthetised, spontaneously hypertensive rats. Eur. J. Pharmacol. 155: 201-203.

Yakimura, T., Unger, Th., Rascher, W., Lang, R.E. and Ganten, D. (1981). Central peptidergic stimulation in blood pressure control: role of enkephalins in rats. Clin. Sci. 61 (Suppl.): 347.

Yamamura, H.I., Gee, K.W., Brinton, R.E., Davis, T.P., Hadley, M. and Wamsley, J.K. (1983). Light microscopic autoradiographic visualisation of [^{3}H]-arginine vasopressin binding sites in rat brain. Life Sci. 32: 1919-1924.

Yamazoe, M., Shiosaka, S., Shibasaki, T. et al (1984). Distribution of six neuropeptides in the nucleus tractus solitarii of the rat: An immunohistochemical analysis. Neurosci. 13: 1243-1266.

Yanagisawa, M., Inoue, A., Ishikawa, T. et al (1988b). Primary structure, synthesis and biological activity of rat endothelin, an endothelium-derived vasoconstrictor peptide. Proc. Natl. Acad. Sci. 85: 6964-6967.

Yanagisawa, M., Kurihara, H., Kimura, S. et al (1988a). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332: 411-415.

Yanagisawa, M. and Masaki, T. (1989a). Molecular biology and biochemistry of the endothelins. Trends in Pharmacol. Sci. 10: 374-378.

Yanagisawa, M. and Masaki, T. (1989b). Endothelin, a novel endothelium-derived peptide. Biochem. Pharmacol. 38 (12): 1877-1883.

Yoshizawa, T., Kimura, S., Kanasawa, I., Yanagisawa, M. and Masaki, T. (1989a). Endothelin-1 depolarises a ventral root potential in the newborn rat spinal cord. J. Cardiovasc. Pharmacol. 13 (Suppl.5): S216-S217.

Zandberg, P., DeJong, W. and DeWied, D. (1979). Effects of catecholamine-receptor stimulating agents on blood pressure after local application in nucleus tractus solitarius of medulla oblongata. Eur. J. Pharmacol. 55: 43-56.

Zukowska-Grojec, Z., Haas, M. and Bayorh, M.A. (1986). Neuropeptide Y and peptide YY mediate non-adrenergic vasoconstriction and modulate sympathetic responses in rats. Reg. Peptides 15: 99-110.