

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

THE BARORECEPTOR REFLEX EMANATING FROM THE CAROTID SINUS
AND COMMON CAROTID ARTERY OF THE SHEEP

A thesis
presented in partial fulfilment of the requirements
for the degree of
Master of Science
in Physiology at Massey University

Karen T. Ball

1987

Abstract of a Thesis Presented in Partial Fulfilment of the Requirements for the
Degree of Master of Science

THE BARORECEPTOR REFLEX EMANATING FROM THE CAROTID SINUS
AND COMMON CAROTID ARTERY OF THE SHEEP

by Karen T. Ball

The aim of this project was to improve understanding of the role of the common carotid arterial baroreceptor mechanism in controlling peripheral blood pressure in the sheep. The responses to clamping of one or both common carotid arteries were examined under chloralose anaesthesia with the vagus nerves intact and after they had been sectioned.

Unilateral clamping of a common carotid artery immediately reduced the mean blood pressure and pulse pressure in the ipsilateral carotid sinus and raised the peripheral mean blood pressure and pulse pressure. The failure of sinus pressures to show any recovery in the clamped vessel suggests that there was minimal flow through anastomoses into the occluded artery. Bilateral clamping of the common carotid arteries reduced the mean blood pressure within both carotid sinuses to a lower level than unilateral clamping, but raised the peripheral mean blood pressure and pulse pressures to a greater degree. This pressor response was interpreted as being due to the larger population of baroreceptors detecting the low carotid sinus pressures during bilateral occlusion.

To test whether there was a tendency for common carotid arterial clamping at different levels to produce different reflex responses of peripheral blood pressure, the carotid arteries were occluded at the caudal, mid- and cranial cervical levels. There was a trend towards a greater rise in peripheral mean blood pressure during caudal clamping compared with cranial clamping. This too may be due to a larger population of baroreceptors detecting the low carotid sinus and common carotid arterial pressures and suggests baroreceptors are distributed in regions of the common carotid artery caudal to the sinus.

In one third of the sheep, clamping the left common carotid artery caused a greater rise in peripheral mean blood pressure than clamping of the right vessel. Possible reasons for this include the presence of a larger population of baroreceptors in the left artery than the right and differences in the sensitivity of receptors in the two vessels.

The variability of responses to clamping and vagotomy was emphasised by the responses of two sheep in which section of the right vagus nerve totally abolished the reflex response to right common carotid arterial occlusion. Since in these animals neither the size of the baroreceptor population nor its sensitivity appeared to be responsible, a conclusion consistent with the evidence is that the baroreceptors in the vessel were innervated by the recurrent laryngeal or vagus nerves. Overall in the experiments, bilateral vagotomy enhanced the peripheral mean blood pressure and pulse pressure responses to clamping the common carotid arteries in keeping with a loss of the input from the aortic arch and cardio-pulmonary baroreceptors.

Histological evidence of the distribution of sensory areas along the common carotid artery was obtained for three discrete areas (A, B and C). It is suggested that baroreceptors located in the common carotid artery may be less sensitive than those in the carotid sinus region because of the low elastin content and lack of tunica medial thinning at the sites of carotid arterial baroreceptor innervation.

This thesis is dedicated to those persons who thrust challenges upon me, and also those who gave me support; but most of all to those rare and precious individuals who provide both caring and challenge.

ACKNOWLEDGEMENT

I am sincerely grateful to Dr D.H. Carr and Dr H.V. Simpson for suggesting this work and for their guidance and advice. I should also like to thank Professor R. Munford, Dr A.S. Davies and Mr M.J. Birtles in the Physiology and Anatomy Department, and particularly Dr K.R. Lapwood for help with the statistical analysis. I also wish to thank Miss C. Thompson, Mr R.I. Sparksman, Mr P.G. Murphy, Mr M. Ratumaitavoki and Mr R.J. Bunch for technical assistance. I am also very grateful to Mrs M. Ball for her dedicated typing of the manuscript.

TABLE OF CONTENTS

<u>Section</u>	<u>Page</u>
Abstract	ii
Acknowledgement	v
List of Tables	xi
List of Figures	xiii
List of Plates	xv
List of Abbreviations	xvi
Chapter 1 - Introduction	1
Chapter 2 - The Anatomy of the Carotid Bifurcation of the Sheep	2
2.1 Introduction	3
2.2 Materials and Methods	4
2.2.1 Preparation of the Corrosion Cast of The Arteries of the Head and Neck	4
2.2.1.1 Preparation of the Sheep for Resin Injection	4
2.2.1.2 Injection of the Resin	4
2.2.2 Preparation of Specimens for the Gross Anatomical Dissection of the Arterial Branches of the Aorta to the Head and Neck and Nerves of the Carotid Bifurcation of the Sheep	5
2.3 Literature Review and Results	7
2.3.1 Arterial Branching in the Head and Neck	7
2.3.2 Gross Innervation of the Carotid Bifurcation	24
Chapter 3 - The Physiology of the Carotid Sinus and Common Carotid Artery of the Sheep	34
3.1 Introduction	34
3.2 Literature Review	35
3.2.1 Discovery of the Carotid Sinus Baroreceptor Reflex	35
3.2.2 Mechanical Aspects of Carotid Sinus Baroreceptor Stimulation	36

3.2.2.1	Fibers Innervating Slowly Adapting Baroreceptors	36
3.2.2.2	Fibers Innervating Rapidly Adapting Baroreceptors	37
3.2.2.3	Mode of Baroreceptor Stimulation	38
3.2.2.4	Modification of the Mode of Baroreceptor Stimulation	39
3.2.3	Comparison of the Carotid Sinus and Aortic Arch Baroreceptor Reflexes	41
3.2.4	Influence of the Carotid Sinus Baroreceptor Afferents on the Cardiovascular Effector Organs	43
3.2.4.1	Regulation of Vascular Tone by the Carotid Sinus Baroreceptor Reflex	44
3.2.4.1.1	Reflex Effects on Regional Arterial Blood Flow	44
3.2.4.1.2	Reflex Effects on the Venous System	45
3.2.4.2	Regulation of Cardiac Performance by the Carotid Sinus Baroreceptor Reflex	46
3.2.4.2.1	Reflex Effects on Heart Rate	47
3.2.4.2.2	Reflex Effects on Stroke Volume	47
3.2.5	Effects of Anaesthesia on the Carotid Sinus Baroreceptor Reflex	48
3.3	Materials and Methods	50
3.3.1	Anaesthetic Protocol	50
3.3.2	Dissection	50
3.3.2.1	Tracheal Cannula	51
3.3.2.2	Femoral Venous Catheter	51
3.3.2.3	Femoral Arterial Catheter	51
3.3.2.4	Lingual Arterial Catheters	51
3.3.2.5	Vagus Nerve Dissection	52
3.3.2.6	Common Carotid Arterial Dissection	52
3.3.2.7	Carotid Sinus Area Dissection	52
3.3.3	Experimental Protocol	52
3.3.3.1	Series 1 - Bilateral Common Carotid Arterial Clamping Before and After Vagotomy	52
3.3.3.2	Series 2 - Common Carotid Arterial Clamping Before and After Vagotomy	54
3.3.3.3	Series 3 - Common Carotid Arterial Tying at Three Positions Before and After Vagotomy	54
3.3.3.4	Series 4 - Combined Common Carotid Arterial, Occipital Group and External Carotid Arterial Clamping Before and After Vagotomy	55
3.3.4	Data Processing	55
3.4	Results	57

3.4.1	Basal Peripheral Mean Blood Pressure, Pulse Pressure, Heart Rate and Respiratory Rate	57
3.4.2	Experimental Series 1 - Bilateral Common Carotid Arterial Clamping Before and After Vagotomy	61
3.4.3	Experimental Series 2 - Common Carotid Arterial Clamping Before and After Vagotomy	61
3.4.3.1	Effect of Common Carotid Arterial Clamping	61
3.4.3.2	Effect of Vagotomy	65
3.4.4	Experimental Series 3 - Common Carotid Arterial Tying at Three Positions Before and After Vagotomy	65
3.4.5	Experimental Series 4 - Combined Common Carotid Arterial, Occipital Group and External Carotid Arterial Clamping Before and After Vagotomy	67
3.4.5.1	Carotid Sinus Mean Blood Pressure and Pulse Pressure	67
3.4.5.1.1	Unilateral and Bilateral Clamping of the Common Carotid Artery	70
3.4.5.1.2	Unilateral Clamping of a Common Carotid Artery and Contralateral Clamping of the Occipital Group and External Carotid Artery	70
3.4.5.1.3	Bilateral Carotid Sinus Isolation	74
3.4.5.1.4	Successive Bilateral Clamping of the Common Carotid Artery, Occipital Group and External Carotid Artery	74
3.4.5.2	Peripheral Mean Blood Pressure	77
3.4.5.2.1	Clamping Position	77
3.4.5.2.2	Effect of Bilateral Vagotomy on the Clamping Cascade	81
3.4.5.3	Peripheral Pulse Pressure	82
3.4.5.4	Heart Rate	82
3.4.5.5	Respiratory Rate	82
3.4.6	Comparison of the Relative Effects of Unilateral Left and Right Common Carotid Arterial Clamping on the Peripheral Mean Blood Pressure Using the Combined Data from Experimental Series 2 to 4	87
3.5	Discussion	90
3.5.1	Anaesthesia	90
3.5.2	Basal Peripheral Mean Blood Pressure and Pulse Pressure	90
3.5.3	Unilateral and Bilateral Clamping of the Common Carotid Artery	92
3.5.4	Unilateral Left and Right Clamping of the Common Carotid Artery	95

3.5.5	Unilateral Cranial and Caudal Tying of the Common Carotid Artery	95
3.5.6	The Effect of Cervical Arterial Clamping on Collateral Blood Flow	96
3.5.7	The Effect of Vagotomy on the Cervical Arterial Clamping Response	98
Chapter 4 - Histology of the Carotid Sinus and Common Carotid Artery in the Sheep		103
4.1	Introduction	103
4.2	Literature Review	104
4.2.1	Structure of the Arterial Wall	104
4.2.1.1	Structure of the Wall of the Carotid Bifurcation	104
4.2.1.2	Structure of the Wall of the Carotid Bifurcation in the Sheep	107
4.2.2	Innervation of the Carotid Sinus	108
4.2.2.1	The Carotid Sinus Nerve	109
4.2.2.1.1	Depth of the Terminal Nerve Fibers	110
4.2.2.1.2	Innervation of the Carotid Sinus	111
4.2.2.1.3	Morphology of the Baroreceptor Terminals	112
4.2.2.1.4	Innervation of the Carotid Sinus in the Sheep	115
4.2.2.1.5	Morphology of the Baroreceptor Terminals in the Sheep	115
4.2.2.2	The External Carotid Nerve	116
4.2.3	Extent of the Carotid Baroreceptor Zone	117
4.2.3.1	Baroreceptor Innervation of the Common Carotid Artery	117
4.3	Materials and Methods	121
4.3.1	Animal Dissection	121
4.3.2	Section Fixation, Processing and Cutting Procedure	121
4.3.2.1	Paraffin Wax Embedded Sections	121
4.3.2.2	Cryostat Sections	123
4.3.3	Staining, Fluorescent and Immunocytochemical Procedures	123
4.3.3.1	Toluidine Blue Stain	123
4.3.3.2	Verhoeff's Haematoxylin Stain	123
4.3.3.3	Sucrose-Potassium-Phosphate Glyoxylic Acid Fluorescence	124
4.3.3.4	Anti-Neuron Specific Enolase Antibody Immunocytochemistry	124
4.3.4	Microscope and Photographic Equipment	126
4.3.5	Measurement of the Dimensions of the Common Carotid Artery	127
4.4	Results	128
4.4.1	Structure of the Wall of the Carotid Sinus	128
4.4.2	Structure of the Wall of the Common Carotid Artery	129

4.4.3	Innervation of the Carotid Sinus	132
4.4.3.1	Identification of the Carotid Sinus	132
4.4.3.2	Sensory and Vasomotor Innervation of the Carotid Sinus	132
4.4.3.3	Vasomotor Innervation of the Carotid Sinus	132
4.4.4	Innervation of the Common Carotid Artery	137
4.4.4.1	Sensory and Vasomotor Innervation of the Common Carotid Artery	137
4.4.4.2	Vasomotor Innervation of the Common Carotid Artery	137
4.4.4.3	Thickness of the Wall in Nerve and Non-Nerve Fiber Areas of the Common Carotid Artery	141
4.5	Discussion	144
4.5.1	The Carotid Sinus	144
4.5.1.1	Structure of the Wall of the Carotid Sinus	144
4.5.1.2	Innervation of the Carotid Sinus	145
4.5.1.2.1	Sensory Innervation	145
4.5.1.2.2	Vasomotor Innervation	145
4.5.2	The Common Carotid Artery	145
4.5.2.1	Structure of the Wall of the Common Carotid Artery	145
4.5.2.2	Innervation of the Common Carotid Artery	148
4.5.2.2.1	Sensory Innervation	148
4.5.2.2.2	Vasomotor Innervation	150
Chapter 5 - Discussion		152
Appendix 1		158
Appendix 2		161
Appendix 3		164
Bibliography		174

LIST OF TABLES

<u>Table</u>	<u>Page</u>
2.1 Arterial Anastomoses of the Arterial Branches of the Aorta to the Head and Neck	fp25
3.1 Basal Peripheral Mean Blood Pressure, Pulse Pressure, Heart Rate and Respiratory Rate (mean \pm SEM) for each Sheep of Experimental Series 2, 3 and 4	58
3.2 Immediate Change of the Basal Peripheral Mean Blood Pressure (mmHg) upon Vagotomy in each Sheep of Experimental Series 2, 3 and 4	60
3.3 Peripheral Mean Blood Pressure Increase (mean \pm SEM, mmHg) and Summary of Analyses of Variance from Experimental Series 2	62
3.4 Peripheral Pulse Pressure Increase (mean \pm SEM, mmHg) and Summary of Analyses of Variance from Experimental Series 2	63
3.5 Heart Rate Increase (mean \pm SEM, beats/min) and Summary of Analyses of Variance from Experimental Series 2	64
3.6 Peripheral Mean Blood Pressure Increase (mean \pm SEM, mmHg) and Summary of Analyses of Variance from Experimental Series 3	68
3.7 Peripheral Mean Blood Pressure Increase (mean \pm SEM, mmHg) and Results of Paired <u>t</u> -tests for the two Sheep of Experimental Series 3 in which Ipsilateral Common Carotid Arterial Tying and Vagus Nerve Section were Undertaken	69
3.8 Mean Blood Pressure and Pulse Pressure (mean \pm SEM, mmHg) in the Left and Right Carotid Sinuses Prior to Vagotomy in Experimental Series 4	71
3.9 Peripheral Mean Blood Pressure Change (mean \pm SEM, mmHg) from Experimental Series 4	78
3.10 Peripheral Mean Blood Pressure Change - Summary of Analysis of Variance from Experimental Series 4	80
3.11 Peripheral Pulse Pressure Change (mean \pm SEM, mmHg) from Experimental Series 4	83

3.12	Peripheral Pulse Pressure Change - Summary of Analysis of Variance from Experimental Series 4	84
3.13	Heart Rate Change (mean \pm SEM, beats/min) from Experimental Series 4	85
3.14	Heart Rate Change - Summary of Analysis of Variance from Experimental Series 4	86
3.15	Peripheral Mean Blood Pressure Change (mean \pm SEM, mmHg) from Experimental Series 2, 3 and 4 and Results of <u>t</u> -test Analyses of Pooled Data	89
4.1	Processing Schedule for the Shandon, Elliot Automatic Tissue Processor	122
4.2	Characteristics of the Left Common Carotid Arterial Nerve Fiber Areas A, B and C of Sheep Number 6 in Experimental Series 2	138
4.3	Arterial Wall Thickness (mean \pm SEM, μ m) in Nerve Fiber Areas A, B and C and Non-Nerve Fiber Areas of the Left Common Carotid Artery of Sheep Number 6 in Experimental Series 2 and Results of <u>t</u> -test Analyses	143

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
2.1 Arterial Branches of the Aorta to the Neck in Relation to the First Five Cervical Vertebrae, Lateral Aspect	fp 8
2.2 Spinal Branches of the Vertebral Artery in Relation to the Cervical Vertebrae, Dorsal Aspect	fp11
2.3 Arterial Branches of the Aorta to the Head in Relation to the Skull, Lateral Aspect.	fp17
2.4 Gross Innervation of the Carotid Bifurcation, Lateral Aspect	fp26
3.1 Arterial Branches of the Common Carotid Artery in Relation to the Base of the Skull, Lateral Aspect	53
3.2 Peripheral Blood Pressure a. and Left b. and Right c. Carotid Sinus Blood Pressures Prior to and Following Section of the Left and Right Vagi in Sheep Number 4 of Experimental Series 4	fp59
3.3 Peripheral Blood Pressure upon Unilateral Clamping of the Caudal Common Carotid Artery Prior to a., During b. and Following c. Section of the Right Vagus Nerve in Sheep Number 4 of Experimental Series 2	fp66
3.4 Peripheral Blood Pressure a. and Left b. and Right c. Carotid Sinus Blood Pressures upon Unilateral and Bilateral Clamping of the Caudal Common Carotid Artery in Sheep Number 4 of Experimental Series 4	fp72
3.5 Peripheral Blood Pressure a. and Left b. and Right c. Carotid Sinus Blood Pressures upon Unilateral Clamping of the Left Common Carotid Artery and Contralateral Clamping of the Right Occipital Group and External Carotid Artery in Sheep Number 4 of Experimental Series 4	fp73
3.6 Peripheral Blood Pressure a. and Left b. and Right c. Carotid Sinus Blood Pressures upon Bilateral Carotid Sinus Isolation in Sheep Number 4 of Experimental Series 4	fp75
3.7 Peripheral Blood Pressure a. and Left b. and Right c. Carotid Sinus Blood Pressures upon Successive Bilateral Clamping of the Caudal Common Carotid Artery, Occipital Group and External Carotid Artery in Sheep Number 4 of Experimental Series 4	fp76

3.8	Diagrammatic Summary of the Significant Contrasts from the Peripheral Mean Blood Pressure Analysis of Variance of Experimental Series 4	fp79
4.1	Schematic Illustration of the Carotid Bifurcation in the Human and Sheep	106
4.2	Type I Baroreceptor from the Wall of the Carotid Sinus of an Adult Man, Tangential Section	fp114
4.3	Type II Baroreceptor from the Wall of the Carotid Sinus of an Adult Man, Tangential Section	114
4.4	The Distribution of Baroreceptors in the Right Common Carotid Artery of the Rabbit, Cat and Dog	119
4.5	The Anti-Neuron Specific Enolase Antibody Immunocytochemical Method	fp125
4.6	A Composite Diagram of the Nerve Axon Locations in Seven Carotid Sinuses at the Level of the Origin of the Occipital Artery from the Common Carotid Artery	134
4.7	The Distribution of Baroreceptors along the Left Common Carotid Artery in Sheep Number 6 of Experimental Series 2	139
4.8	Measurement Sites in the Wall of the Left Common Carotid Artery	142

LIST OF PLATES

<u>Plate</u>	<u>Page</u>
4.1 Transverse Section of the Left Common Carotid Artery at the Level of the Origin of the Occipital Artery Demonstrating the Structural Modification of the Left Carotid Sinus	fp130
4.2 Transverse Section of the Right Common Carotid Artery at the Level of the Origin of the Occipital Artery Demonstrating the Structural Modification of the Right Carotid Sinus	fp130
4.3 Transverse Section of the Left Common Carotid Artery 35 mm from the Origin of the Occipital Artery Demonstrating the Structure of Nerve Fiber Area A	fp131
4.4 Transverse Section of the Left Common Carotid Artery 35 mm from the Origin of the Occipital Artery Demonstrating the Structure Adjacent to Nerve Fiber Area A	fp131
4.5 Transverse Section of the Left Common Carotid Artery at the Level of the Origin of the Occipital Artery Demonstrating the Appearance of Vascular Smooth Muscle	fp133
4.6 Transverse Section of the Left Common Carotid Artery at the Level of the Origin of the Occipital Artery Demonstrating the Appearance of the Left Carotid Sinus Terminal Nerve Fibers	fp135
4.7 Transverse Section of the Right Common Carotid Artery at the Level of the Origin of the Occipital Artery Demonstrating the Appearance of the Right Carotid Sinus Terminal Nerve Fibers	fp135
4.8 Transverse Section of the Left Parotid Salivary Gland Demonstrating the Adrenergic Innervation of a Capillary	fp136
4.9 Transverse Section of the Left Common Carotid Artery at the Level of the Origin of the Occipital Artery Demonstrating the Sparse Adrenergic Innervation of the Left Carotid Sinus	fp136
4.10 Transverse Section of the Medio-Adventitial Border of the Left Common Carotid Artery Demonstrating the Terminal Nerve Fibers of Area A	fp140
4.11 Transverse Section of the Medio-Adventitial Border of the Left Common Carotid Artery Demonstrating the Terminal Nerve Fibers of Area A	fp140

LIST OF ABBREVIATIONS

A	Arterial
BSA	Bovine serum albumin
CCA	Common carotid artery
Cd	Caudal position (middle of fifth cervical vertebra)
Cn	Cranial position (cranial pole of second cervical vertebra)
DAB	Diaminobenzidine
ECA	External carotid artery
fp	Facing page
G	Ganglion
Int	Intact
L	Left
Md	Mid position (caudal pole of third cervical vertebra)
N	Nervus
n	Number of animals, unless otherwise stated
NFA	Nerve fiber area
NNFA	Non-nerve fiber area
No.	Number
NSE	Neuron specific enolase
OG	Occipital group
PBS	Phosphate buffered saline
R	Right
SPG	Sucrose-potassium-phosphate glyoxylic acid
Vn	Vagus nerve

Only simpletons find things absolutely clear.

Alexander Solzhenitsyn
August 1914

CHAPTER 1

INTRODUCTION

The concept that the cardiovascular system is regulated by neural reflexes originating from the great vessels and heart is more than 100 years old, but its importance was probably not fully appreciated until Hering discovered the carotid sinus baroreceptor reflex in 1923. Subsequently, intense study of this subject has led to the present recognition of the central role of the arterial baroreflexes in circulatory control.

Baroreceptors are stretch receptors predominantly located in the adventitia of the carotid sinus and aortic arch, and the frequency of firing of these receptors varies directly with both the mean blood pressure and the rate of change of blood pressure. Afferent signals pass to nuclei in the floor of the fourth ventricle, where, by a system of interneurons, an increase in baroreceptor impulses results in reflex inhibition of sympathetic adrenergic efferents to the cardiovascular system and reflex stimulation of the cardiac vagus nerve, leading to a decrease in systemic pressure. Baroreceptors are tonically active when blood pressure is normal and, therefore, a decrease in blood pressure causes a reduction of baroreceptor impulses and a rise in blood pressure to its normal level (Kircheim, 1976).

Heymans and Neil (1958) commented that "the temporary and incomplete loss of baroreceptor activity caused by carotid occlusion causes such an obvious hypertension that it is used all over the world to demonstrate the sinus reflexes to students". This is the case at Massey University, where the dog was initially the subject of the physiology student. During an acute experiment on these animals, clamping of both the left and right common carotid arteries caused regular and reproducible moderate increases in blood pressure and heart rate. In addition, bilateral section of the vagus nerves could be relied on to enhance these responses significantly. These results are similar to those observed by other workers in this animal.

Difficulty in obtaining dogs caused the Physiology Department to substitute the sheep in this experiment. The change of species presented a major problem: unusual cardiovascular responses began to emerge from this experiment. In particular, sheep displayed poor blood pressure and heart rate rises upon bilateral common carotid arterial occlusion, a significant fall in basal blood pressure upon section of both the left and right vagus nerves and the failure of bilateral vagotomy to enhance the clamping response.

Since such atypical observations do not appear to have been reported in other species, the present project was instigated in order to explain these findings and improve the knowledge of the basic mechanisms involved in the control of blood pressure in sheep. This initially involved an anatomical review of the arterial supply of blood to the ovine cephalic circulation. The students' physiological observations were then examined and repeated under more suitable conditions of anaesthesia and further clamping protocols were subsequently undertaken in an attempt to clarify the carotid sinus baroreceptor mechanism in the sheep. The results from these latter experiments were suggestive of baroreceptors down the length of the common carotid artery and, because of this, the final component of this project was to study histologically the innervation of the ovine common carotid artery.