Western University
Scholarship@Western

Digitized Theses | Digitized Special Collections

1993

Control Of Tonic Sympathetic Nerve Activity By The Brainstem In Rats

Kristen Hayes

Follow this and additional works at: https://ir.lib.uwo.ca/digitizedtheses

Recommended Citation

Hayes, Kristen, "Control Of Tonic Sympathetic Nerve Activity By The Brainstem In Rats" (1993). *Digitized Theses*. 2376.
https://ir.lib.uwo.ca/digitizedtheses/2376

This Dissertation is brought to you for free and open access by the Digitized Special Collections at Scholarship@Western. It has been accepted for inclusion in Digitized Theses by an authorized administrator of Scholarship@Western. For more information, please contact tadam@uwo.ca, wlswadmin@uwo.ca.

CONTROL OF TONIC SYMPATHETIC NERVE ACTIVITY BY THE BRAINSTEM IN RATS

by

Kristen Hayes

Department of Physiology

Submitted in partial fulfilment

of the requirements for the degree of

Doctor of Philosophy

Faculty of Graduate Studies

The University of Western Ontario

London, Ontario

October, 1992

© Kristen Hayes 1993

National Library of Canada

Bibliothèque nationale du Canada

Acquisitions and Bibliographic Services Branch

Direction des acquisitions et des services bibliographiques

395 Wellington Street
Ottawa, Ontario
K1A 0N4

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file Votre référence

Our file Notre référence

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-90573-5

Canada

Abstract

Control of tonic firing of sympathetic nerves by the rostral ventrolateral medulla (RVLM) and the pontine reticular formation (PRF) was investigated. Electrical activity of renal, splenic, mesenteric and preganglionic greater splanchnic nerves and T_{13} white rami was recorded in artificially respired, urethane-anaesthetized rats. Glycine (1.0M;180nl) was microinjected into the RVLM to inhibit neuronal discharge and effects on these nerves were compared. To study descending inhibitory influences on these nerves, effects of RVLM blockade were compared to effects of cervical spinal cord transection. RVLM blockade caused greater decreases in discharge of renal (-44 ±3%) than splenic (-31 ±2%) nerves, had no effect on mesenteric nerves and decreased discharge of T_{13} white rami (-34 ±8%) more than that of splanchnic nerves (-18 ±4%). RVLM blockade and cord transection caused similar decreases in splanchnic (-34 ±5% and -32 ±5%) and splenic (-45 ±6% and -48 ±7%) nerve firing. However, RVLM blockade caused greater decreases than cord transection in discharge of renal nerves (-53 ±5% vs. -27 ±2%) and T_{13} white rami (-34 ±8% vs. +12 ±28%). These findings demonstrate that neurons controlling the kidney depend more on excitation from the RVLM than do those controlling the spleen and intestine and that renal neurons may also receive tonic descending inhibition.

Next, glycine (1.0M;65nl) was injected into the PRF to locate regions providing tonic control of arterial pressure, heart rate and activity of renal and splenic nerves in artificially respired, Saffan-anaesthetized rats. PRF blockade caused large, short-lasting decreases in arterial pressure and equal decreases in renal (-47 ±4%) and splenic (-45

±4%) nerve activity. PRF blockade also inhibited ongoing firing of 10 RVLM cardiovascular neurons, so characterized because their discharge was inhibited by baroreceptor activation and was synchronized to the cardiac cycle. These findings indicate that PRF neurons contribute to resting control of vasomotor discharge and provide tonic excitation to RVLM cardiovascular neurons. Finally, the anterograde tracer *Phaseolus vulgaris leucoagglutinin* (PHA-L) was iontophoresed into PRF sites in which glycine injections caused cardiovascular responses. PHA-L-labelled axons and terminals were identified primarily within the medullary reticular formation and RVLM. Therefore, PRF neurons could influence tonic sympathetic outflow by synaptic connections in either of these regions. The generation of sympathetic nerve activity probably includes an interaction between the PRF and RVLM.

This thesis is dedicated to Dr. Marilyn Robinson:

finder of gold nuggets.

Acknowledgements

I am indebted to Dr. Lynne Weaver for her undeviating enthusiasm, support and guidance. I wish to thank those people who contributed to some of these studies: Vijaya Chevendra for her technical help with the retrograde tracing technique, Dr. Chris Yardley for completing some spinal cord transection experiments, Dr. Andrei Krassioukov for completing some experiments for a dose-response curve for glycine, Dr. Franco Calaresu and Dr. Sandeep Agarwal for their advice and assistance with the extracellular recordings of medullary neurons and Dr. Gary Allen and Dr. Dave Cechetto for their advice throughout the *Phaseolus* anterograde tracing research project. I also wish to thank the members of my advisory committee, Drs. Robert Kline, Dave Cechetto and Franco Calaresu. Finally, a very special thanks to Beverly Hayes, to Jean Hugues Martin and to all of my family and friends for their encouragement and support.

Table of Contents

Page

Certificate of Examination ii

Abstract iii

Dedication v

Acknowledgements vi

Table of Contents vii

List of Tables xi

List of Figures xii

Introduction 1

Chapter 1: Selective control of sympathetic pathways to the kidney, spleen and intestine by the ventrolateral medulla in rats. 6

1.1 Introduction 6

1.1.1 Search for a cardiovascular centre 6

1.1.2 The rostral ventrolateral medulla 7

1.1.3 Characteristics of RVLM neurons that control tonic sympathetic nerve activity 7

1.1.4 Differential control of sympathetic nerve activity by the RVLM . . . 11

1.2 Rationale 12

1.3 Methods 14

Preparation of animals 14

Neural recordings 15

Microinjection procedures 18

Experimental protocol 18

Statistical analysis 20

1.4 Results 22

1.4.1 Sympathetic responses to stimulation of arterial baroreceptors 22

1.4.2 Sympathetic responses to unilateral blockade of the rostral ventrolateral medulla 22

1.4.3 Simultaneous recordings from renal and splenic nerves 36

1.4.4 Control injections 36

1.4.5 Sympathetic responses to RVLM blockade in rats anaesthetized with alpha- chloralose 36

1.4.6 Histological verification of injection sites 37

1.5 Discussion 40

Chapter 2: Pre- and postganglionic sympathetic activity in white rami of rats 47
2.1 Introduction 47
2.2 Methods and Results 48
2.2.1 Electrophysiological experiments 48
2.2.2 Histological observations 54
2.3 Conclusions and discussion 57

Chapter 3: Evidence for descending tonic inhibition specifically affecting sympathetic pathways to the kidney in rats 60
3.1 Introduction 60
3.3.1 Tonic inhibition of sympathoexcitatory neurons in the rostral ventrolateral medulla 60
The caudal ventrolateral medulla 60
The raphe nuclei 63
3.1.2 Tonic bulbospinal inhibition of sympathetic activity 65
3.1.3 Supraspinal neurons responsible for tonic descending inhibition 67
The raphe nuclei 67
The A5 cell group 68
3.2 Rationale 70
3.3 Methods 72
Preparation of animals 72
Cervical spinal cord transection 73
Bilateral microinjections of glycine into the rostral ventrolateral medulla 74
Unilateral microinjections of glycine into the region of the A5 cell group 75
Experimental protocol 76
Statistical analysis 77
3.4 Results 78
3.4.1 Sympathetic responses to stimulation of arterial baroreceptors 78
3.4.2 Sympathetic responses to cervical spinal cord transection 78
3.4.3 Bilateral microinjections of glycine into the rostral ventrolateral medulla 83
3.4.4 Comparison of cervical spinal cord transection and RVLM blockade 88
3.4.5 Unilateral microinjections of glycine into the A5 region 88
3.5 Discussion 93

Chapter 4: Tonic sympathetic excitation and vasomotor control from pontine reticular neurons in rats 99
4.1 Introduction 99
4.2 Rationale 103
4.3 Methods 104
Surgical procedures 104
Neural recordings 105
Microinjection procedures 106
Statistical analysis 108

4.4 Results 110
4.4.1 Sympathetic responses to stimulation of baroreceptors 110
4.4.2 Microinjections of glycine into the pontine reticular formation 110
4.4.3 Microinjections of glycine into the rostral ventrolateral medulla 115
4.4.4 Microinjections of NaCl and sucrose into the pontine reticular formation 122
4.4.5 Microinjections of D,L-homocysteic acid into the pontine reticular formation 123
4.4.6 Baroreceptor denervation 123
4.4.7 Bilateral microinjections of glycine into the pontine reticular formaton 127
4.4.8 Microinjections of muscimol into the pontine reticular formation 128
4.4.9 Histological verification of injection sites 128
4.5 Discussion 132

Chapter 5: Pontine reticular neurons provide tonic excitation to neurons in the rostral ventrolateral medulla in rats 138
5.1 Introduction 138
5.2 Methods 140
Surgical procedures 140
Recordings from renal nerves 141
Recordings from RVLM units 142
Microinjection procedures 143
Experimental protocol 143
Histology 145
Statistical analysis 146
5.3 Results 147
5.4 Discussion 160

Chapter 6: Axonal projections of pontine reticular neurons involved in tonic cardiovascular control identified with the anterograde tracer *Phaseolus vulgaris leucoagglutinin* in rats 165
6.1 Introduction 165
6.2 Methods 167
Surgical procedures 167
Microinjection procedures 167
Anterograde tracing procedures 168
Immunocytochemistry 169
Statistical analysis 170
6.3 Results 171
6.3.1 Anatomy of the pontine reticular formation 171
6.3.2 Cardiovascular responses to glycine injection into the pontine reticular formation 171
6.3.3 Anterograde labelling of Phaseolus vulgaris leucoagglutinin - injection sites 174

32. Brightfield and darkfield photomicrographs showing PHA-L labelled axons and terminals within the parabrachial nucleus and nucleus tractus solitaris . . . 184

33. Series of camera lucida-like drawings of transverse sections of rat pons, medulla, cervical spinal cord and first thoracic spinal cord illustrating the injection site and [illegible] of labelled fibres and putative termination fields of a PHA-L injection into the region of the A5 cell group 186

List of Tables

Table	Description	Page

1. Effect of unilateral microinjection of glycine (1.0M) into the rostral ventrolateral medulla on arterial pressure and heart rate. 28
2. Effect of ganglionic blockade with chlorisondamine on electrical activity of 13th thoracic white rami. 53
3. Sympathetic and cardiovascular responses to microinjection of D,L-homocysteic acid into the pontine reticular formation. 124

List of Figures

Figure	Description	Page

1. View of the 13th thoracic ganglion and its rostral and caudal connecting rami after retraction of the psoas muscle 17

2. Mean arterial pressure and renal nerve responses to unilateral microinjections of muscimol into the rostral ventrolateral medulla 24

3. Arterial pressure and renal and splenic nerve responses to a unilateral microinjection of glycine into the rostral ventrolateral medulla of one rat . 26

4. Mean responses of renal, splenic, mesenteric, preganglionic greater splanchnic and preganglionic 13th thoracic white ramus sympathetic nerves to unilateral injections of glycine into the rostral ventrolateral medulla 30

5. Comparison of maximal responses of renal, splenic, mesenteric, preganglionic greater splanchnic and preganglionic 13th thoracic white ramus sympathetic nerves to unilateral blockade of the rostral ventrolateral medulla with glycine 32

6. Mean responses of greater splanchnic nerves and 13th thoracic white rami to unilateral injections of glycine into the rostral ventrolateral medulla before and after ganglionic blockade 35

7. Drawings of transverse sections of rat medulla showing sites of glycine injection 39

8. Neurograms of sympathetic discharge from the 13th thoracic connecting ramus in rat no. 890419 during a control period and following ganglionic blockade with chlorisondamine 52

9. Photomicrographs of a single thionin-stained neuron with two nucleoli and a ganglion-like cluster of thionin-stained neurons within a 13th thoracic connecting ramus bundle 56

10. Arterial pressure and renal and splenic nerve responses of one rat to cervical spinal cord transection 80

11. Mean responses of renal, splenic, preganglionic greater splanchnic and preganglionic 13th thoracic white ramus sympathetic nerves to cervical spinal cord transection 82

12. Mean responses of renal, splenic and greater splanchnic nerves to bilateral injections of glycine into the rostral ventrolateral medulla 85

13. Comparison of neural responses to cervical spinal cord transection with responses to blockade of the rostral ventrolateral medulla 87

14. Arterial pressure, heart rate and renal and splenic nerve responses of one rat to a unilateral microinjection of glycine into a site near the A5 cell group . 90

15. Drawings of transverse sections of rat pons line showing sites of glycine injection in the vicinity of the A5 cell group 92

16. Arterial pressure, heart rate and renal and splenic nerve responses to a unilateral microinjection of glycine into the pontine reticular formation of one rat 112

17. Mean changes in mean arterial pressure and heart rate and renal and splenic nerve activity caused by unilateral injections of glycine into the pontine reticular formation 114

18. The relationship between the concentration of glycine and the mean changes in mean arterial pressure, heart rate and renal nerve activity caused by injections into the pontine reticular formation 117

19. Arterial pressure, heart rate and renal and splenic nerve responses to a unilateral injection of glycine into the rostral ventrolateral medulla of one rat 119

20. Mean responses of mean arterial pressure and heart rate and renal and splenic nerve activity to unilateral injections of glycine into the rostral ventrolateral medulla 121

21. Arterial pressure, heart rate and renal and splenic nerve responses to a unilateral injection of D,L-homocysteic acid into the pontine reticular formation of one rat 126

22. Location of pontine and medullary sites of glycine injection 131

23. Identification of a cardiovascular unit in the rostral ventrolateral medulla . 149

24. Arterial pressure, heart rate, renal nerve and RVLM cardiovascular unit responses to a unilateral microinjection of glycine into the pontine reticular formation of one rat 151

25. Identification and response of a unit located in the region of the rostral ventrolateral medulla to glycine injection into the pontine reticular formation 154

26. Identification and response of a non-cardiovascular unit located in the region of the rostral ventrolateral medulla to glycine injection into the pontine reticular formation 157

27. Drawings of transverse sections of rat pons and medulla showing locations of sites of injection and recording 159

28. Cardiovascular responses to a unilateral microinjection of glycine into the pontine reticular formation of one rat 173

29. Brightfield photomicrographs showing a site of injection of PHA-L into the pontine reticular formation and anterograde labelling of axons and terminals within the medullary reticular formation, rostral ventrolateral medulla and intermediolateral cell column of the thoracic spinal cord 176

30. Series of drawings of transverse sections of rat pons showing the location and diameters of the PHA-L injections into the pontine reticular formation and surrounding areas 178

31. Series of camera lucida-like drawings of transverse sections of rats pons, medulla, cervical and thoracic spinal cord illustrating the injection site and course of labelled fibres and the putative termination fields of a representative PHA-L injection into the pontine reticular formation 181

32. Brightfield and darkfield photomicrographs showing PHA-L labelled axons and terminals within the parabrachial nucleus and nucleus tractus solitaris . . . 184

33. Series of camera lucida-like drawings of transverse sections of rat pons, medulla, cervical spinal cord and first thoracic spinal cord illustrating the injection site and course of labelled fibres and putative termination fields of a PHA-L injection into the region of the A5 cell group 186

The author of this thesis has granted The University of Western Ontario a non-exclusive license to reproduce and distribute copies of this thesis to users of Western Libraries. Copyright remains with the author.

Electronic theses and dissertations available in The University of Western Ontario's institutional repository (Scholarship@Western) are solely for the purpose of private study and research. They may not be copied or reproduced, except as permitted by copyright laws, without written authority of the copyright owner. Any commercial use or publication is strictly prohibited.

The original copyright license attesting to these terms and signed by the author of this thesis may be found in the original print version of the thesis, held by Western Libraries.

The thesis approval page signed by the examining committee may also be found in the original print version of the thesis held in Western Libraries.

Please contact Western Libraries for further information:
E-mail: libadmin@uwo.ca
Telephone: (519) 661-2111 Ext. 84796
Web site: http://www.lib.uwo.ca/

Introduction

An essential feature of the sympathetic nervous system is the presence of "a continuous flow of impulses" from the central neurons of this system to peripheral neurons and finally to effector tissues (Polosa, Mannard & Laskey, 1979). This "tonic" or "background" activity can be recorded as action potential discharge in sympathetic neurons and their axons (Adrian, Bronk & Phillips, 1932; Mirgorodsky & Skok, 1969; Coote & Westbury, 1979) and is critical for homeostasis including maintenance of arterial blood pressure within the normal range. The fall in total peripheral resistance following ganglionic blockade clearly demonstrates the importance of tonic sympathetic nerve activity in maintaining vascular smooth muscle tone (Iriuchijima & Sakata, 1985). Tonic activity in sympathetic neurons also contributes to the precise regulation of vasomotor tone because the discharge rate in sympathetic nerves can be either increased or decreased and because by "priming" effector cells, neurogenic tone increases the rate of responsiveness of vascular smooth muscle to changes in sympathetic input (Polosa *et al.*, 1979). Supraspinal neurons appear to have an important role in generating sympathetic nerve activity since transection of the neuraxis near the medullospinal junction causes an immediate and profound fall in arterial pressure and sympathetic activity to some vascular beds (Alexander, 1946; Ardell, Barman & Gebber, 1982). Although the genesis of tonic sympathetic nerve activity has fascinated researchers since its discovery (Bernard (1851) and Brown-Séquard (1852), as cited in: Gebber, 1990), the mechanism and location of its origin remains a question that is hotly debated but still unanswered today.

Contrary to Cannon's (1929) description of generalized sympathetic activation, the

distribution of tonic activity is not uniform to sympathetic nerves innervating different organs. For example, in conscious rats, administration of a ganglionic blocking drug causes substantial decreases in resistance of renal and carotid vascular beds but not in superior mesenteric and hindquarter vascular beds (Iriuchijima & Sakata, 1985). These different changes of vascular resistances following ganglionic blockade probably reflect differences in the magnitude of sympathetic vasoconstrictor tone. Differential control of sympathetic nerve activity may be mediated through specific brainstem nuclei. Spinal cord transection in cats and rats reduces efferent activity in some but not all sympathetic nerves. Discharge of postganglionic renal, gastric, hepatic, adrenal and lumbar chain sympathetic nerves is substantially decreased in spinal cats while firing of splenic, mesenteric and greater splanchnic nerves is maintained (Meckler & Weaver, 1985; Qu, Sherebrin & Weaver, 1988). After cervical spinal cord transection in rats, lumbar sympathetic nerve activity is reduced significantly but discharge of renal and splenogastric nerves is increased (Taylor & Schramm, 1987; 1988). In addition, although two different sympathetic nerves (i.e. renal and inferior cardiac) recorded simultaneously show a 2- to 6-Hz activity pattern, the predominant 2- to 6-Hz frequency component in the basal discharge of one sympathetic nerve is different from that in a second nerve (Barman, Gebber & Calaresu, 1984). Since the 2- to 6-Hz activity originates in the brainstem (Gebber, 1990), separate networks of brainstem neurons may control tonic activity in different sympathetic nerves. In fact, some brainstem neurons have been shown to be differentially correlated to different postganglionic nerves (Barman *et al.*, 1984).

Identifying the cell groups in the brainstem that are involved in regulation of the

sympathetic nervous system has been approached using several different methods. Retrograde and anterograde tracing substances have located those brainstem neurons that project directly to the thoracolumbar intermediolateral cell column (IML) of the spinal cord, a region which contains most of the sympathetic preganglionic neurons (Cabot, 1990). More recently, neurotropic viruses such as herpes simplex type I and herpes virus suis (pseudorabies) have been used as transynaptic markers of central autonomic pathways from specific target tissues (Strack, Sawyer Platt & Loewy, 1989a; Strack, Sawyer, Hughes, Platt & Loewy, 1989b; Schramm, Strack, Platt & Loewy, 1991; Dehal, Dekaban, Krassioukov, Picard & Weaver, 1992; Li, Ding, Wesselingh & Blessing, 1992a). These anatomical techniques are often combined with immunocytochemical staining for neurotransmitters, peptides and their synthesizing enzymes. Tract-tracing studies have not identified vasomotor neurons antecedent to those that project to the IML and also do not provide information about the functional role of these cell groups.

Microinjection of excitatory amino acids into specific regions of the brainstem has provided information about the functional role of these cell groups in controlling vasomotor activity (Goodchild, Dampney & Bandler, 1982). However, since silent neurons as well as those that are tonically active are excited, groups of neurons involved in generation of tonic sympathetic discharge must be identified by methods that eliminate their ongoing discharge. Brainstem areas can be destroyed by electrolytic lesions but sympathetic effects of these lesions could result from the destruction of axons of neurons remote from the vicinity of the lesion. Microinjection of inhibitory chemicals such as glycine or gama-aminobutyric acid (GABA) inhibits the ongoing activity of neuronal cell

bodies and dendrites without affecting axons of passage and is one technique for studying the tonic effects of a group of neurons on sympathetic nerve activity.

Single-unit extracellular recording experiments have attempted to identify medullary neurons that are part of sympathetic networks. Neurons that respond to activation of baroreceptor afferent nerves may be involved in cardiovascular regulation. However, without other identifying criteria this approach has marginal significance because many neurons which control non-autonomic functions may also respond to baroreceptor activation (Barman & Gebber, 1981). In addition to responding to activation of baroreceptors, the same neurons should also have a naturally occurring discharge that is correlated to the cardiac cycle because baroreceptor afferent nerves impart vasomotor neurons with a strongly pulse-synchronous pattern of discharge (Brown & Guyenet, 1985; Barman & Gebber, 1985). Finally, medullary neurons may be tested for discharge that correlates with sympathetic nerve discharge (Barman & Gebber, 1988). This technique assumes that central neurons with tonic activity correlated with the 2- to 6-Hz slow wave of sympathetic discharge contribute to the generation of sympathetic outflow. The disadvantage of this method used alone is that neurons that are not involved in vasomotor control but that share common inputs with central sympathetic networks are also identified. Neurons with all three properties are likely to be part of central networks controlling sympathetic activity, although this conservative approach probably excludes many vasomotor neurons.

The present studies have used microinjection, single-unit extracellular recording and neuroanatomical tract-tracing methods to provide information about brainstem neurons

that are important in tonic cardiovascular and sympathetic regulation. An emphasis has been placed upon the presence of selective sympathetic influences on individual visceral organs such as the kidney, spleen and intestine.

Chapter 1: Selective control of sympathetic pathways to the kidney, spleen and intestine by the ventrolateral medulla in rats.

Journal of Physiology (London) 428: 371-385, 1990.

1.1 Introduction

1.1.1 Search for a cardiovascular centre

The most important neural factor in determining resting levels of arterial blood pressure is the ongoing discharge of spinal vasoconstrictor neurons. The search for the group or groups of supraspinal neurons responsible for generating this basal sympathetic activity has continued for more than 100 years. Early experiments by Dittmar (1870) and Owsjannikow (1871) demonstrated that the medulla and the pons are essential for maintaining resting levels of arterial blood pressure since transection of the brainstem caudal to the inferior colliculus had no effect on blood pressure but subsequent caudal transections produced increasingly larger decreases in arterial pressure (as cited in: Gebber, 1984). Alexander (1946) later demonstrated that transection of the neuraxis at the level of the obex caused reductions in arterial pressure comparable to those seen following cervical spinal cord transection. The fall in arterial pressure was accompanied by the cessation of inferior cardiac sympathetic nerve activity. These studies indicated that the crucial centres for neurogenic support of blood pressure were located somewhere in the medulla. Later, it was suggested that the critical neurons responsible for generating vasomotor tone were not concentrated within a single brainstem area but rather were dispersed along the neuraxis (Hilton, 1975; Hilton & Spyer, 1980). This conclusion was

based on evidence that electrical stimulation of several supramedullary regions produced increases in arterial pressure and differential changes in regional blood flows (Hilton, 1979).

1.1.2 The rostral ventrolateral medulla

This view has been overshadowed by evidence supporting the concept that a specific group of neurons in the rostral portion of the ventrolateral medulla (RVLM) is the most critical site for cardiovascular control and for generation of tonic excitatory drive to sympathetic vasomotor neurons. Attention was initially focused on the RVLM as a source of vasomotor tone when a "glycine-sensitive area" was localized on the ventral surface of the medulla by Feldberg and Guertzenstein (1972; 1976), Guertzenstein (1973) and Guertzenstein and Silver (1974). Bilateral electrolytic lesions or topical application of pentobarbital or the inhibitory amino acid glycine to this region caused decreases in arterial pressure similar to those observed after spinal cord transection. These studies provided the first evidence that the ongoing activity of neurons in the ventrolateral medulla is important for the support of resting arterial pressure.

1.1.3 Characteristics of RVLM neurons that control tonic sympathetic nerve activity

The role of the RVLM in tonic sympathetic control has since been studied extensively and has been reviewed in several recent articles (Calaresu & Yardley, 1988; Barman, 1990; Guyenet, 1990; McCall, 1990; Chalmers & Pilowsky, 1991). Since electrolytic lesions cannot distinguish between cell bodies and fibres of passage, recent studies have used specific neurotoxins (i.e. kainic acid or tetrodotoxin) or inhibitory amino acids (i.e. glycine or gamma-aminobutyric acid (GABA) to selectively destroy cell bodies or inhibit

neuronal activity, respectively, within the RVLM. Destruction of neurons in the RVLM or inhibition of their ongoing activity reduced arterial pressure and sympathetic nerve activity to the levels observed after spinal cord transection (Ross, Ruggiero, Joh, Park & Reis, 1984; Granata, Ruggiero, Park, Joh & Reis, 1985a; Pilowsky, West & Chalmers, 1985; Dean & Coote, 1986; Granata, Numao, Kumada & Reis, 1986; Willette, Punnen-Grandy, Krieger & Sapru, 1987; Stein, Weaver & Yardley, 1989; Yardley, Stein & Weaver, 1989a; Beluli & Weaver, 1991b) indicating that the tonic discharge of these neurons maintains vasomotor tone.

Electrophysiological studies have provided strong evidence that RVLM neurons have tonic sympathoexcitatory functions. Neurons have been identified in this region that exhibit spontaneous discharges temporally correlated with the cardiac cycle and with the 2- to 6-Hz slow wave of sympathetic activity (Brown & Guyenet, 1984; 1985; Barman & Gebber, 1983; 1985; Morrison, Milner & Reis, 1988). After baroreceptor afferent fibres have been sectioned, RVLM neuronal activity loses its cardiac-related rhythmicity but remains correlated with sympathetic discharge indicating that RVLM neurons are part of a descending pathway that controls sympathetic activity (Barman & Gebber, 1983; Morrison *et al.*, 1988). The ongoing activity of RVLM neurons is inhibited by baroreceptor activation (Dembowsky & McAllen, 1990) and antidromic mapping studies reveal that axons of these neurons terminate in the intermediolateral cell column (IML; Brown & Guyenet, 1984; 1985; Barman & Gebber, 1985; Morrison *et al.*, 1988; Sun, Young, Hackett & Guyenet, 1988a; Chan, Chan & Wong, 1991) and central autonomic nucleus (Caverson, Ciriello & Calaresu, 1983) of the spinal cord, regions known to

contain sympathetic preganglionic neurons (Cabot, 1990). In addition, Sun, Hackett and Guyenet (1988b) demonstrated in rats that some neurons in the RVLM develop pacemaker-like activity after intracisternal administration of the glutamate receptor antagonist, kynurenate and that the regular discharge pattern of RVLM neurons in medullary slices arises from typical pacemaker potentials (Sun *et al.*, 1988a). These "pacemaker" neurons may be responsible for generating sympathetic nerve discharge.

The population of sympathoexcitatory neurons described by single-unit recordings and by microinjection and lesion experiments is located in a discrete area that includes the medulla at a rostrocaudal level between the rostral two thirds of the inferior olives and the caudal pole of the facial nucleus. This region is bounded dorsally by the nucleus ambiguus and medially by the inferior olive. There is a close overlap between the location of the RVLM pressor area and the C1 group of adrenergic neurons suggesting that the bulbospinal C1 neurons mediate cardiovascular responses from the RVLM (review: Reis, Morrison & Ruggiero, 1988; Ruggiero, Cravo, Arango & Reis, 1989). However, the pacemaker neurons described by Sun *et al.* (1988) are not catecholaminergic (Sun, Young, Hackett & Guyenet, 1988c). Neurons within this discrete area contain a wide variety of neurotransmitters and peptides including serotonin (Sasek, Wessendorf & Helke, 1990), acetylcholine (Milner, Pickel, Giuliano & Reis, 1989), met- and leu-enkephalin (Sasek & Helke, 1989), somatostatin (Finley, Maderdrut, Roger & Petrusz, 1981), neurotensin (Ciriello, Caverson & Polosa, 1986), neuropeptide Y (Chronwall, Di Maggio, Massari, Pickel, Ruggiero & O'Donohue, 1985; Blessing, Oliver, Hodgson, Joh & Willoughby, 1987), cholecystokinin (Mantyh & Hunt, 1984), vasoactive intestinal

peptide (Leibstein, Dermietzel, Willenberg & Pauschert, 1985), angiotensin II (Krukoff, Vu, Harris, Aippersbach & Jhamandas, 1992), galanin (Krukoff *et al.*, 1992), thyrotropin-releasing hormone (Sasek *et al.*, 1990), substance P (Sasek *et al.*, 1990) and glutamate (Minson, Pilowsky, Llewellyn-Smith, Kaneko, Kapoor & Chalmers, 1991) and anatomical tracing studies have confirmed that many of these neurons project to the IML. In particular, compelling evidence has been presented for the involvement of glutamate in tonic descending control of sympathetic preganglionic neurons (review: McCall, 1990). When the excitatory amino acid antagonist kynurenic acid was infused intrathecally (Guyenet, Sun & Brown, 1987) or microiontophoresed directly onto sympathetic preganglionic neurons (Morrison, Ernsberger, Milner, Callaway, Gong & Reis, 1989) sympathoexcitation evoked by electrical stimulation of the RVLM was eliminated. Similarly, intrathecal administration of a substance P antagonist prevented the pressor response elicited by microinjection of excitatory amino acids into the RVLM suggesting that substance P may also function as an excitatory transmitter of RVLM neurons (review: Loewy, 1987; McCall, 1990).

The contribution of the RVLM to tonic cardiovascular control is not completely understood. In some circumstances the importance of the RVLM to control of tonic sympathetic nerve activity may change. For example, extensive bilateral lesions of the RVLM in anaesthetized rats produced only transient (30 minutes) decreases in arterial pressure and the magnitude and duration of this response was anaesthetic-dependent. Cardiovascular responses to RVLM lesions were larger and longer-lasting in urethan-anaesthetized rats than in rats anaesthetized with either alpha-chloralose or sodium

pentobarbital (Cochrane, Buchholz, Hubbard, Keeton & Nathan, 1988). Furthermore, bilateral electrolytic or neurotoxic RVLM lesions in conscious rats did not affect basal arterial pressure or heart rate and did not eliminate the baroreflex cardiac response to a rise in arterial pressure (Cochrane & Nathan, 1989; Vasquez, Lewis, Varner & Brody, 1992).

1.1.4 Differential control of sympathetic nerve activity by the RVLM

The RVLM does not produce equal activation of all components of sympathetic outflow. McAllen (1986) showed that microinjection of excitatory amino acids into the RVLM causes pressor responses, peripheral vasoconstriction and increases in the activity of cervical, splanchnic and inferior cardiac nerves. Since excitation of RVLM pressor neurons has no effect on pupils, nictitating membranes, piloerection or intestinal motility (McAllen, 1986), the RVLM may specifically affect vasomotor neurons. Furthermore, not all sympathetic vasomotor nerves have equal dependence on excitatory drive from the RVLM. In cats, inhibiting the discharge of RVLM neurons by bilateral microinjections of the inhibitory amino acid glycine, or by application of glycine to the surface of the ventral medulla elicited greater reductions in renal than in mesenteric nerve activity (Stein *et al.*, 1989; Yardley *et al.*, 1989a). In addition, the spontaneous activity of some ventrolateral medullary neurons is differentially related to the discharge of different postganglionic nerves. For example, the activity of one RVLM unit may be more strongly correlated to renal nerve activity than to inferior cardiac nerve activity or vice versa (Barman *et al.*, 1984). These observations suggest that even tonic excitatory influences from the RVLM to different sympathetic nerves are distributed in a non-

uniform pattern.

This extensive collection of evidence indicates that the rostral ventrolateral medulla is an important region in cardiovascular control although may not be essential for the maintenance of tonic sympathetic activity supporting arterial pressure.

1.2 Rationale for the present study

The purpose of the present investigation was to determine if the RVLM, in the rat, has selective tonic influences on certain components of lower thoracic and upper lumbar sympathetic outflow. The specific goals were to determine if inhibition of tonic activity (blockade) of neurons in the RVLM causes differential responses in postganglionic renal, splenic and mesenteric nerves. Since anatomical and electrophysiological evidence suggests that renal nerves receive most of their preganglionic input from 10th thoracic to first lumbar (T_{10}-L_1) white rami and that splenic and mesenteric neurons are innervated primarily by the greater splanchnic nerve (Chevendra & Weaver, 1991; Taylor & Weaver, 1992a) responses of preganglionic greater splanchnic nerves and T_{13} white rami to RVLM blockade were compared to those of postganglionic renal, splenic and mesenteric nerves. Ongoing activity of RVLM neurons was inhibited with microinjections of the inhibitory amino acid glycine or the $GABA_A$ agonist muscimol. Glycine reduces neuronal firing by activating the inhibitory glycine receptor, a chloride channel protein that is abundant in the spinal cord and brainstem of vertebrates. Activation of the glycine receptor results in an increase in chloride permeability and hyperpolarization of the neuronal membrane (Werman, Davidoff & Aprison, 1968; Young & Snyder, 1973; Aprison & Daly, 1978;

Betz & Becker, 1988; Sato, Zhang, Saika, Sato, Tada & Tohyama, 1991). Activation of $GABA_A$ receptors which are located throughout the central nervous system inhibits neuronal activity by a similar effect on membrane permeability to chloride (DeFeudis, 1990). Finally, because one study reported that microinjection of muscimol into the RVLM of rats anaesthetized with alpha-chloralose failed to decrease renal sympathetic nerve activity (Poree & Schramm, 1989), the influence of two different anaesthetic agents, urethane and alpha-chloralose, on the cardiovascular and neural responses to RVLM blockade was also compared.

1.3 Methods

Preparation of animals.

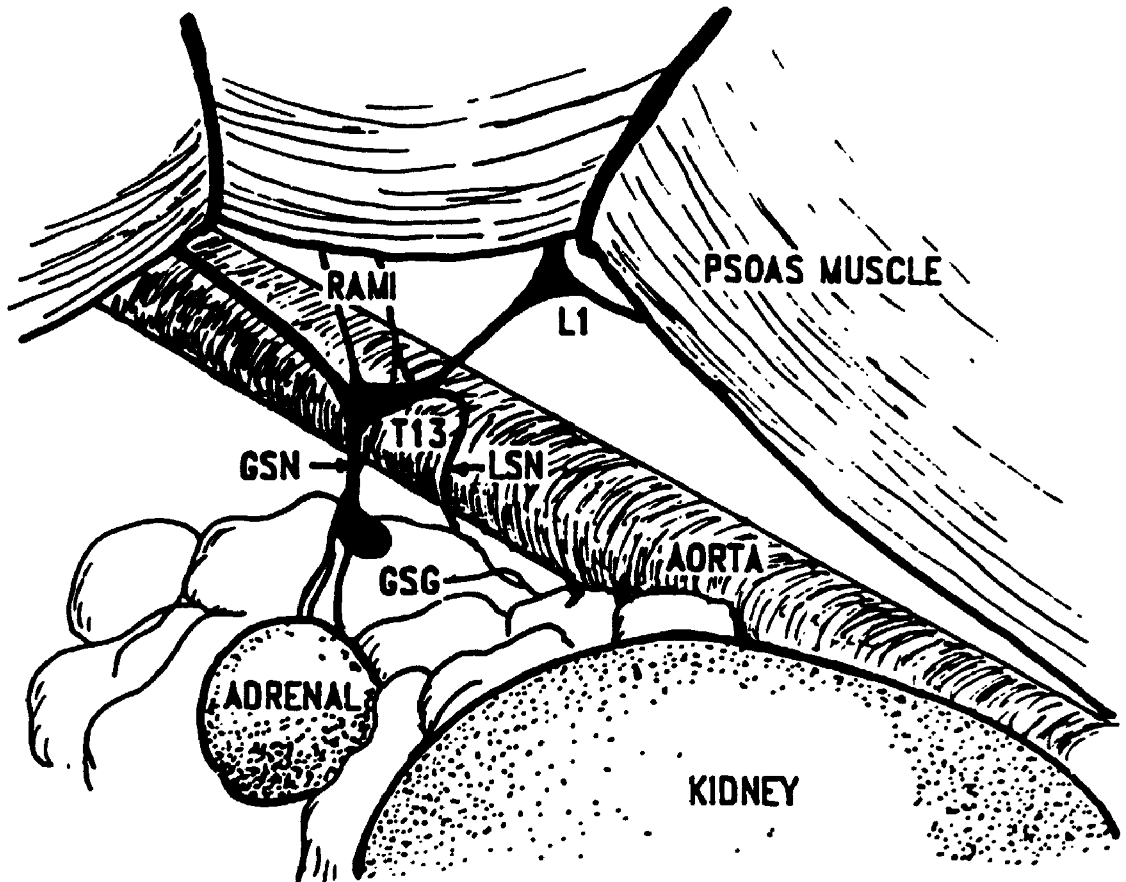
Male Wistar rats (200-400 g; Charles River, Canada) were anaesthetized with urethane (1.4 g/kg, i.p.; Aldrich Chemical Company, USA) and tracheal tubes were inserted. The left jugular vein and the right femoral artery were cannulated for the delivery of solutions and drugs and for monitoring arterial blood pressure, respectively. Arterial blood samples (~100 μl) were withdrawn periodically for analysis of pH, pO_2 and pCO_2 (pH/blood gas analyser, model 170, Corning Medical, USA) and acid-base disorders were corrected by i.v. administration of sodium bicarbonate or by adjusting respiratory rate and/or tidal volume. Body temperature was maintained at 37°C with a heating pad. A solution of physiological saline was infused intravenously (0.6 ml/hr) throughout the experiment to compensate for fluid loss. The animals were artificially respired with oxygen-enriched air and paralysed with gallamine triethiodide (20 mg/kg, i.v.; Rhone-Poulenc, Canada). Supplemental doses of gallamine (10 mg/kg, i.v.) were given when spontaneous respiratory movements were observed. Before each supplemental dose of gallamine was given, the animal's plane of anaesthesia was assessed by examining palpebral and withdrawal reflexes. Additional urethane (0.1 g/kg, i.v.) was given if needed.

The rats were placed in a stereotaxic frame (David Kopf Instruments, USA) and a section of the interparietal bone was removed to expose the cerebellum overlying the medulla. The exposed surface of the brain was kept moist with saline-soaked gauze until immediately before injection of glycine.

Neural recordings.

The left sympathetic chain, the right anterior bundle of the greater splanchnic nerve or postganglionic renal and splenic nerves were exposed via a retroperitoneal approach. The mesenteric nerve was approached through a ventral midline incision. The anatomy of the thoracolumbar chain region as observed in these experiments is shown in Fig. 1. To expose the T_{10}-L_1 white rami, the psoas muscle was retracted laterally by sutures placed around the muscle and the sympathetic chain was isolated. The greater splanchnic nerve was identified and followed rostrally to its junction with the sympathetic chain. This junction was used as a landmark for identifying specific ganglia as the greater splanchnic nerve leaves the sympathetic chain at the 13th sympathetic ganglion (T_{13}; Zeigler, 1988). The diaphragm was detached from the chest wall to permit adequate exposure of the T_{13} ganglion. The rostral and caudal T_{13} white rami were identified and one of the two was selected randomly, dissected free from surrounding tissue and its distal end severed (Fig. 1). Small bundles of all other sympathetic nerves were also identified, dissected free from surrounding tissue and severed. The central ends were placed on stainless-steel bipolar electrodes for recording multifibre electrical activity. Exposed nerves were covered with dental impression medium (Regasil, Dentsply International Inc., U.S.A.) to isolate the nerve and electrode from the surrounding tissue and to prevent dehydration. In all animals a pneumothorax was made to eliminate artifacts in the neural recordings caused by respiratory movements. Neural discharge was amplified at a bandwidth of 30 Hz to 3 kHz. The amplified signals were monitored on an oscilloscope, recorded on magnetic tape and the integrated neural signals were

Figure 1. View of the 13th thoracic ganglion (T_{13}) and its rostral and caudal connecting rami after retraction of the psoas muscle. L_1, first lumbar paravertebral ganglion; GSN, greater splanchnic nerve; LSN, lesser splanchnic nerve; GSG, greater splanchnic ganglion. Adapted from Zeigler (1988) with permission.


RAMI
L1
PSOAS MUSCLE
T13
GSN
LSN
GSG
AORTA
ADRENAL
KIDNEY

displayed with arterial pressure and heart rate on a Grass Instruments polygraph (Grass Instrument Company, U.S.A.) In 4 experimer.., discharge of postganglionic renal and splenic nerves was recorded simultaneously.

To ensure that recordings were from sympathetic nerves containing vasomotor components, arterial baroreceptors were stimulated by increasing arterial pressure with a bolus injection of 1-3 μg phenylephrine (i.v., Neo-Synephrine, Sterling, Canada) to elicit decreases in heart rate and sympathetic nerve activity. All postganglionic nerves tested exhibited baroreceptor sensitivity.

Microinjection procedures.

Glass micropipettes were pulled to a tip size of about 40 μm and filled with a solution of the inhibitory amino acid, glycine (1.0 M, BDH Chemicals, Canada), in distilled water (pH =7.4). A few drops of India ink were added to 10 ml of the solution to mark the injection site in the brain. The tips of the pipettes were positioned in the rostral ventrolateral medulla (RVLM) according to the stereotaxic atlas of Paxinos and Watson (1986) and glycine (100-180 nl) was injected unilaterally by application of pressurized pulses of air to the pipette. Ejection pressure and pulse duration were controlled by a picospritzer (General Valve Corporation, U.S.A.). Injection volumes were determined by the displacement of the meniscus at the air-liquid interface in the pipette observed through a microscope containing an ocular micrometer.

Experimental protocol.

Arterial pressure, heart rate and discharge of sympathetic nerves were recorded from 1 min before to 5 min after the beginning of microinjection of glycine into the RVLM.

A recovery recording was taken when nerve discharge and arterial pressure had returned to control values. At some of these sites the same volume of a solution of sodium chloride (1.0 M) was injected into the site before the injection of glycine. At other sites the inhibitory actions of the gamma-aminobutyric acid ($GABA_A$) agonist muscimol (0.001 M, Fluka Chemical Corporation, USA) were tested.

Since preganglionic splanchnic nerves and white rami bundles may contain some postganglionic fibres (Celler & Schramm, 1981; Hayes, Chevendra & Weaver, 1990a), a ganglionic blocking drug, hexamethonium (Sigma Chemical Company, USA) or chlorisondamine (Ciba Geigy Ltd., Canada), was given to eliminate postganglionic nerve activity. Once a site was found in which glycine produced an arterial pressure and sympathetic nerve response, a bolus injection of hexamethonium (30mg/kg, i.v.) or chlorisondamine (5mg/kg, i.v.) was given. In order to maintain arterial pressure at levels comparable to those before ganglionic blockade, a constant infusion of phenylephrine (0.1-0.2 μg/min of 0.2 mg/ml in saline) was begun and continued for the duration of the experiment. Glycine was injected into the same site to observe neural responses of the preganglionic fibres only.

In 3 experiments, rats were anaesthetized with halothane (Halocarbon Laboratories Inc., USA) followed by alpha-chloralose (100 mg/kg, i.v., Sigma Chemical Company, USA) and prepared surgically as outlined above. Supplemental doses of chloralose (10-25 mg/kg) were given as needed. Recordings of renal sympathetic nerve activity were obtained, tested for baroreceptor sensitivity and then glycine was injected (180 nl) unilaterally into the RVLM.

At the end of the experiments, the animals were given an overdose of urethane and background electrical noise was recorded for 2 min. The brains were removed and stored in a solution of 10% Formalin in saline. Transverse sections of 50 μm were cut on a cryostat, stained with neutral red dye and the sites of injection were verified using a Leitz microscope (Wild-Leitz, Canada).

Neural discharge of all postganglionic nerves and preganglionic splanchnic nerves was recorded on magnetic tape (Racal, model 7DS, UK) digitized, rectified and integrated cumulatively during 10 s periods. After subtracting integrated background electrical noise, integrated nerve activity was expressed in μV·s. White ramus activity was quantified in counts of spikes/s after window discrimination (Frederick Haer, U.S.A.) because after ganglionic blockade, these nerve bundles have only a few active fibres firing (Hayes *et al.*, 1990a). Spike counting was considered appropriate because integrated values of neural activity are greatly affected by the amplitudes of single spikes in few-fibre preparations (Meckler & Weaver, 1985) and changes cannot be detected when voltages are very small. The thresholds for window discrimination were set very close to the maximum amplitude of background electrical noise during meticulous inspection of nerve activity monitored on an oscilloscope and by an audio monitor to exclude background noise and to include as much nerve activity as possible in the quantification procedures. Values of mean arterial blood pressure are presented throughout this study.

Statistical Analysis

A one-way analysis of variance (ANOVA) with repeated measures was used to determine statistical changes in neural discharge and arterial pressure during stimulation

of baroreceptors and after injection of solutions of glycine, muscimol or sodium chloride into the RVLM (Snedecor & Cochran, 1980). Comparison between percent changes in arterial pressure and nerve activity between groups was made using a one-way ANOVA and the Student Newman-Keuls test after square root normalization of percentage values. Differences were considered significant when $p<0.05$ and variability was expressed as a pooled standard error derived from ANOVA, a coefficient of variation or as a standard error of the mean.

1.4 Results

1.4.1 Sympathetic responses to stimulation of arterial baroreceptors

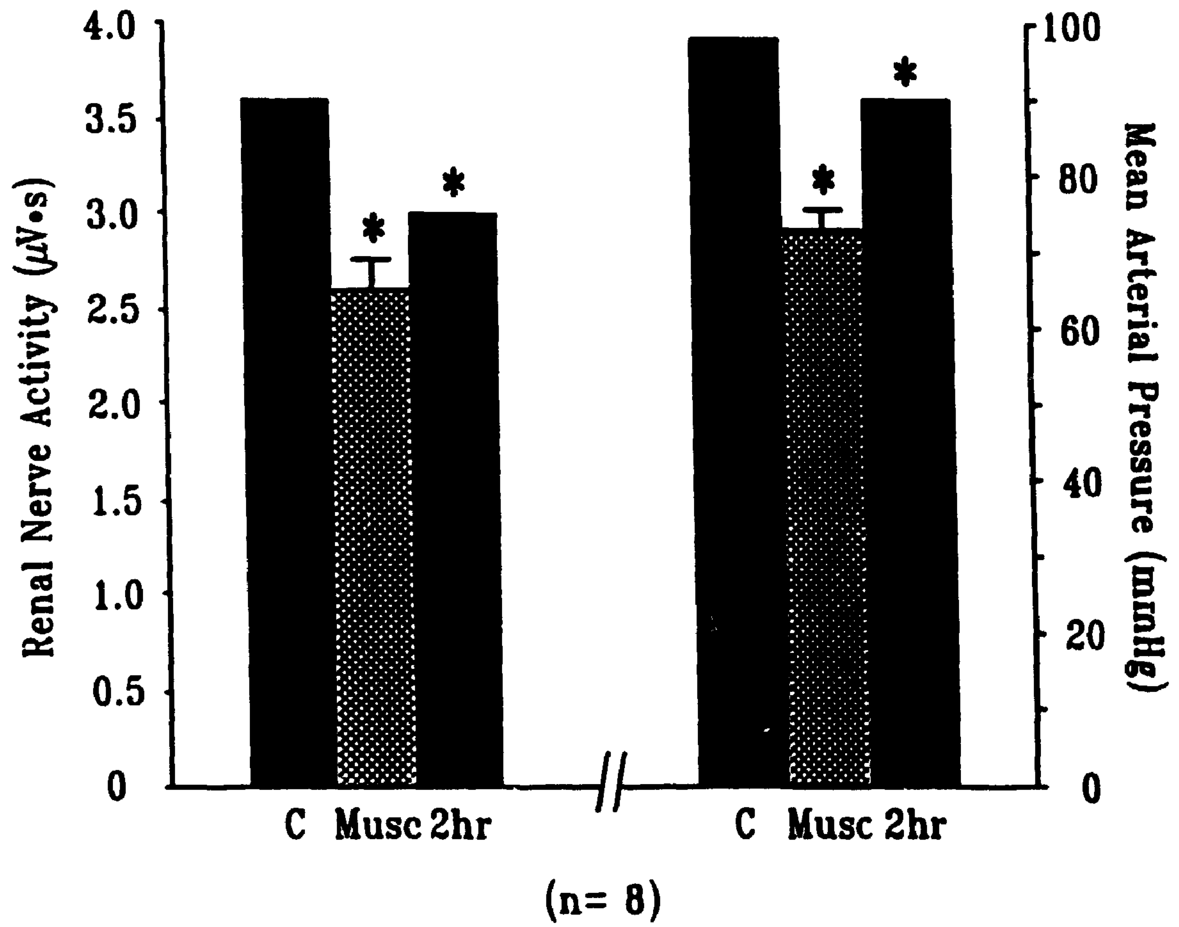
All postganglionic nerves were tested for pressoreceptor sensitivity by evaluating responses to increases in arterial pressure elicited by i.v. injections of phenylephrine. An increase in mean arterial pressure from 85 ±4 to 152 ±5 mmHg significantly inhibited ongoing discharge of renal nerves (n=13) by 76 ±7% (from 3.6 to 0.7 μV·s; pooled S.E.=0.46), splenic nerves (n=8) by 62 ±7% (from 3.9 to 1.3 μV·s; pooled S.E.=0.62) and mesenteric nerves (n=4) by 22 ±3% (from 4.5 to 3.5 μV·s; pooled S.E.=0.21). The decrease in renal nerve activity was significantly greater than the decrease in splenic nerve activity and discharge of both renal and splenic nerves was inhibited more than that of mesenteric nerves.

1.4.2 Sympathetic responses to unilateral blockade of the RVLM

The actions of the inhibitory $GABA_A$ agonist muscimol were tested by unilateral injections into the RVLM. Eight injections (35-180 nl) of muscimol in 6 rats lowered arterial pressure by 25 ±2% and renal nerve activity was reduced by 31 ±4% (Fig. 2). Arterial pressure and renal nerve activity did not return to control values even 2 hrs after microinjection of muscimol. Since the cardiovascular and neural responses to muscimol were so long-lasting, the inhibitory amino acid, glycine, was used to inhibit activity of neurons in the RVLM in all subsequent experiments.

An example of the sympathetic and arterial pressure response produced by a unilateral injection of glycine (180 nl) into the RVLM is shown in Fig. 3. Glycine injection produced a large decrease in arterial pressure as well as changes in nerve activity that

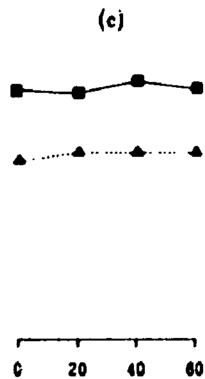
Figure 2. Mean arterial pressure and renal nerve responses to unilateral microinjections (35-180 nl) of muscimol into the RVLM of urethane-anaesthetized rats. The solid bars are average discharge during 60 s control (C) and 2 hrs after muscimol injection (2 hr). The cross-hatched bars are discharge during the 10 s of maximum response to muscimol (Musc). Variability is indicated by the pooled standard error and asterisks indicate significant difference from control. Number of injections (n =8). Muscimol caused significant decreases in mean arterial pressure and nerve activity that did not return to control values 2 hrs after injection.

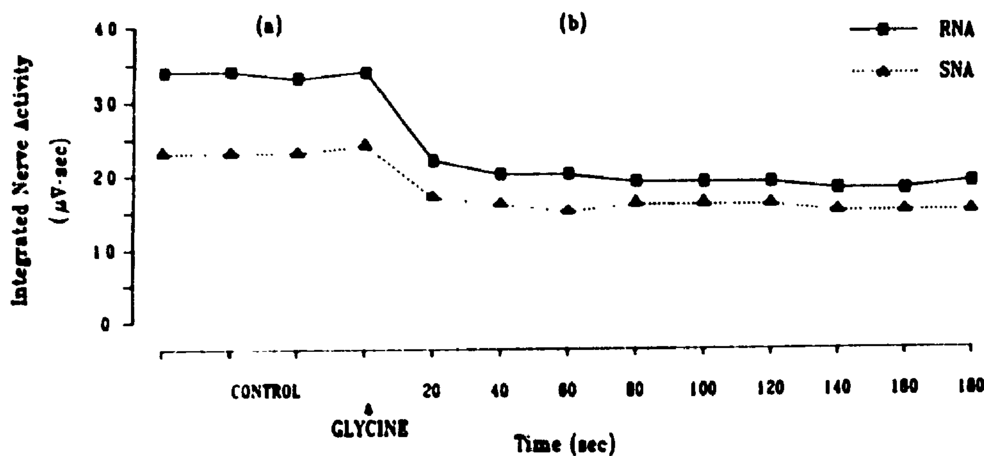


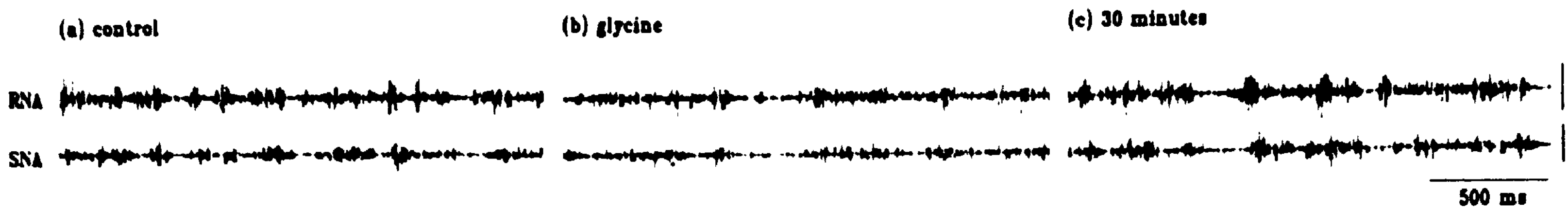
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Renal Nerve Activity (μV•s)
100
80
60
40
20
0
Mean Arterial Pressure (mmHg)
*
C Musc 2hr
C Musc 2hr
(n= 8)

Figure 3. Responses to a unilateral microinjection (180 nl) of glycine (1.0 M) into the RVLM of one urethane-anaesthetized rat. A: record of systemic arterial blood pressure is illustrated in the top panel. Integrated activity from renal (■) and splenic (Δ) nerves (recorded simultaneously) is illustrated in the second panel. Time is indicated beneath these panels. B: renal and splenic sympathetic nerve activity (RNA and SNA) before (a) and 60 s (b) and 30 min (c) after glycine injection. Vertical calibration is 100 μV. Glycine injection produced a large decrease in blood pressure and a decrease in renal nerve discharge that was greater than the decrease in splenic nerve discharge.

A
Blood Pressure
(mmHg)
150
100
50
0


30 minutes


(a)
(b)
(c)
RNA
SNA
Integrated Nerve Activity
(μV·sec)
40
30
20
10
0
CONTROL
GLYCINE
20
40
60
80
100
120
140
160
180
Time (sec)
0
20
40
60


B
(a) control
(b) glycine
(c) 30 minutes
RNA
SNA
500 ms

began within seconds and reached a maximum decrease within 80 s. The change in renal nerve activity was larger than the change in splenic nerve activity. Arterial pressure and nerve activity returned to control values within 30 min. Maximum decreases always occurred within 1-3 min of injection and arterial pressure and nerve activity returned to control values within 30 min to 1 hr. Mean arterial pressure and heart rate for all animals (n=40) were reduced significantly from 95 ±3 mmHg to 61 ±2 mmHg and from 473 ±5 to 447 ±6 bpm, respectively, following glycine injection. The mean arterial pressure and heart rate responses during renal, splenic, mesenteric, greater splanchnic and white ramus nerve recordings are shown in Table 1. Since the arterial pressure responses to glycine during each of these nerve recordings were not statistically different, glycine injections appeared to have produced a similar blockade of the RVLM affecting the activity of all the 5 nerves studied.

Nineteen injections of glycine in 13 rats decreased renal nerve activity by 44 ±3% and 11 injections in 8 rats caused decreases in splenic nerve activity of 31 ±2% (Fig. 4). Glycine injection did not produce consistent responses in mesenteric nerves (Fig. 4). Five injections in 4 rats caused mesenteric nerve activity to increase by 11 ±4%. However, another 2 injections in 2 of these rats caused nerve activity to decrease 19 ±3%. The mean 3 ±6% increase in mesenteric nerve activity in these animals was not significant. The decrease in renal nerve activity was significantly greater than the decrease in splenic nerve activity (Fig. 5). Both renal and splenic sympathetic responses were significantly different from the response of the mesenteric nerve.

Since greater splanchnic nerves and white rami contain some postganglionic fibres,

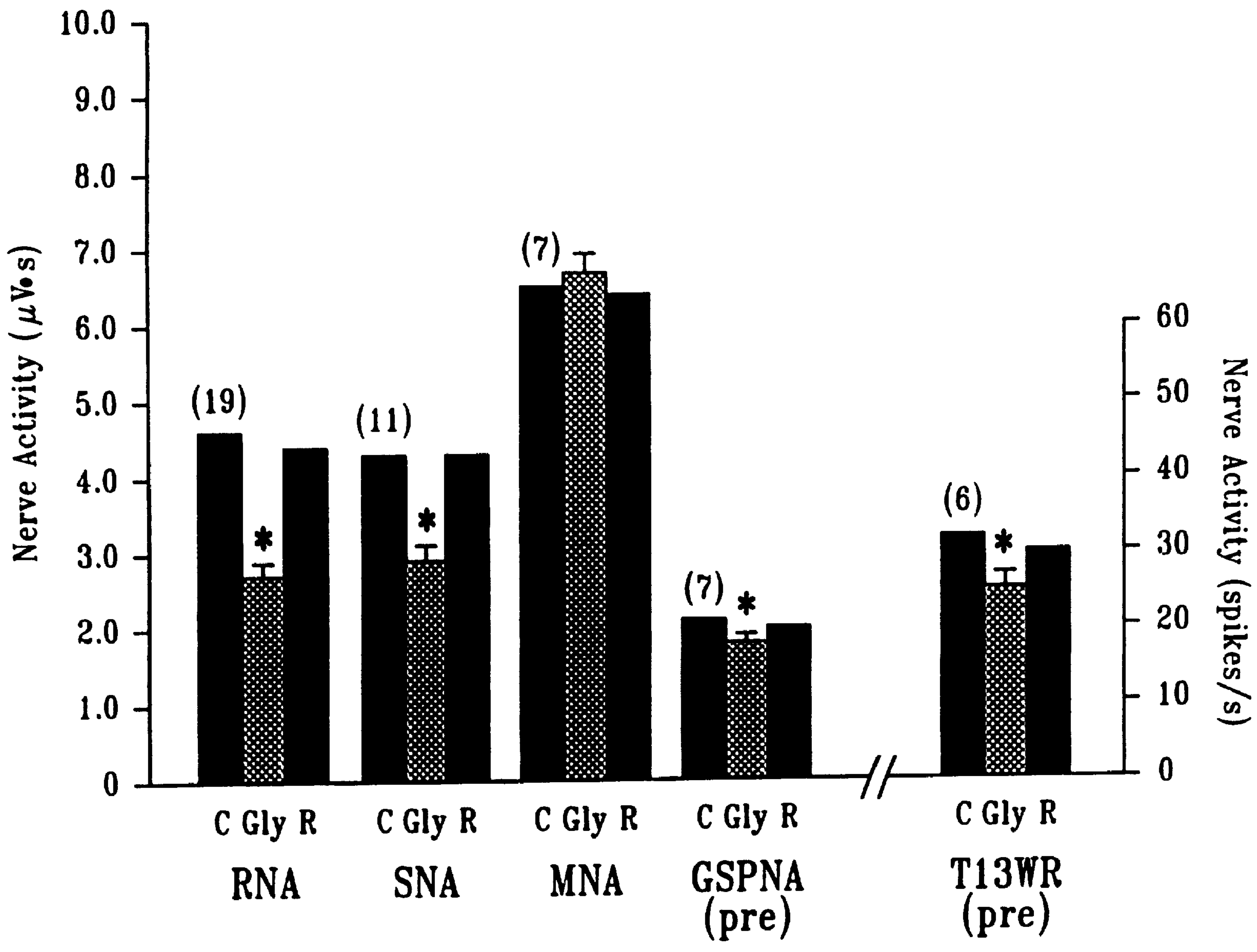
Table 1. Effect of unilateral microinjection of glycine (1.0M) into the RVLM on arterial pressure and heart rate.

	Control	Maximum Decrease	Recovery	Change	Pooled S.E.	Percent Change
Arterial Pressure (mmHg)						
Renal (19)	96	60 *	91	36	2.1	37 ± 2
Splenic (11)	101	66 *	90 *	35	2.3	34 ± 3
Mesenteric (7)	86	59 *	77	27	3.2	31 ± 4
Splanchnic (7)	96	64 *	86	32	3.2	32 ± 3
T13WR (8)	88	55 *	79	33	3.0	37 ± 3
Heart Rate (bpm)						
Renal	462	436 *	464 *	26	2.8	5.8 ± 0.9
Splenic	486	462 *	485 *	24	3.6	5.0 ± 0.9
Mesenteric	466	445	467	21	8.0	4.5 ± 1.8
Splanchnic	496	456 *	491 *	35	5.0	8.3 ± 1.8
T13WR	476	456 *	468	19	4.7	4.0 ± 1.8

* significantly different from control

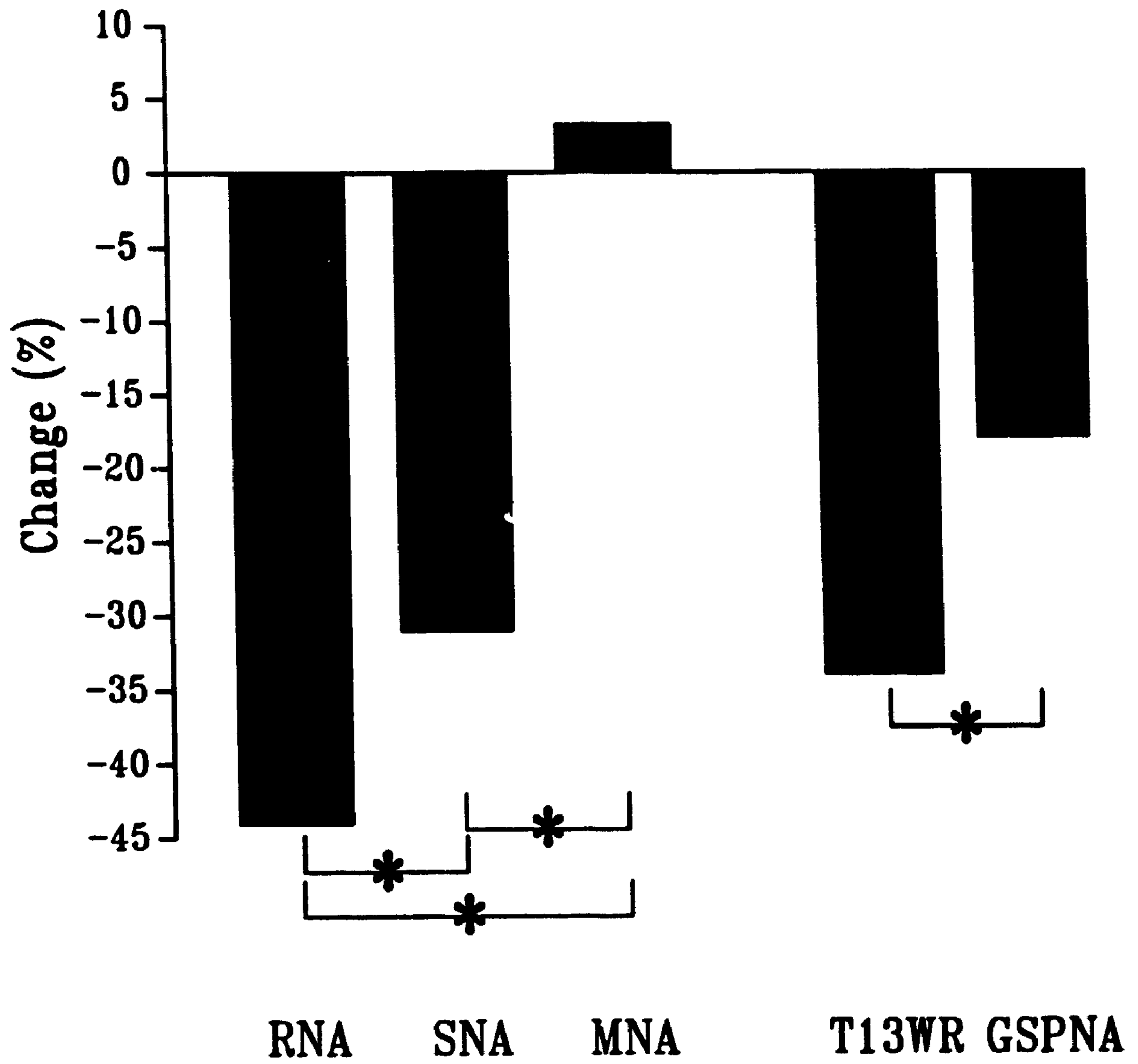
Renal, splenic, mesenteric, splanchnic and T_{13} white rami (WR) indicate groups of animals from which discharge of these nerves was recorded. Splanchnic and T_{13}WR groups were those before ganglionic blockade. Numbers in parentheses indicate number of injections tested.

Figure 4. Mean responses of renal (RNA), splenic (SNA), mesenteric (MNA), preganglionic greater splanchnic (GSPNA) and preganglionic T_{13} white ramus (T_{13}WR) sympathetic nerves to unilateral injections of glycine into the RVLM of urethan-anaesthetized rats. The solid bars are average discharge during 60 s control (C) and recovery (R) periods. The cross-hatched bars are discharge during the 10 s of maximum response to glycine (Gly). Variability is indicated by the pooled standard error and asterisks indicate significant difference from control. Numbers of injections are given in parentheses. Significant decreases in nerve activity following glycine injection were seen in all but mesenteric nerves.



Nerve Activity (µV•s)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0
(19)
(11)
(7)
(7)
(6)
*
*
*
*
C Gly R
C Gly R
C Gly R
C Gly R
C Gly R
RNA
SNA
MNA
GSPNA
(pre)
T13WR
(pre)
Nerve Activity (spikes/s)
60
50
40
30
20
10
0

Figure 5. Comparison of maximal responses to unilateral blockade of the RVLM with glycine. These responses are those illustrated in **Figure 4** expressed as a percentage change from control. Brackets with asterisks indicate significant differences between groups. Coefficient of variation is 0.39. Renal, splenic and mesenteric responses were all different from each other. The preganglionic responses were also different from each other.

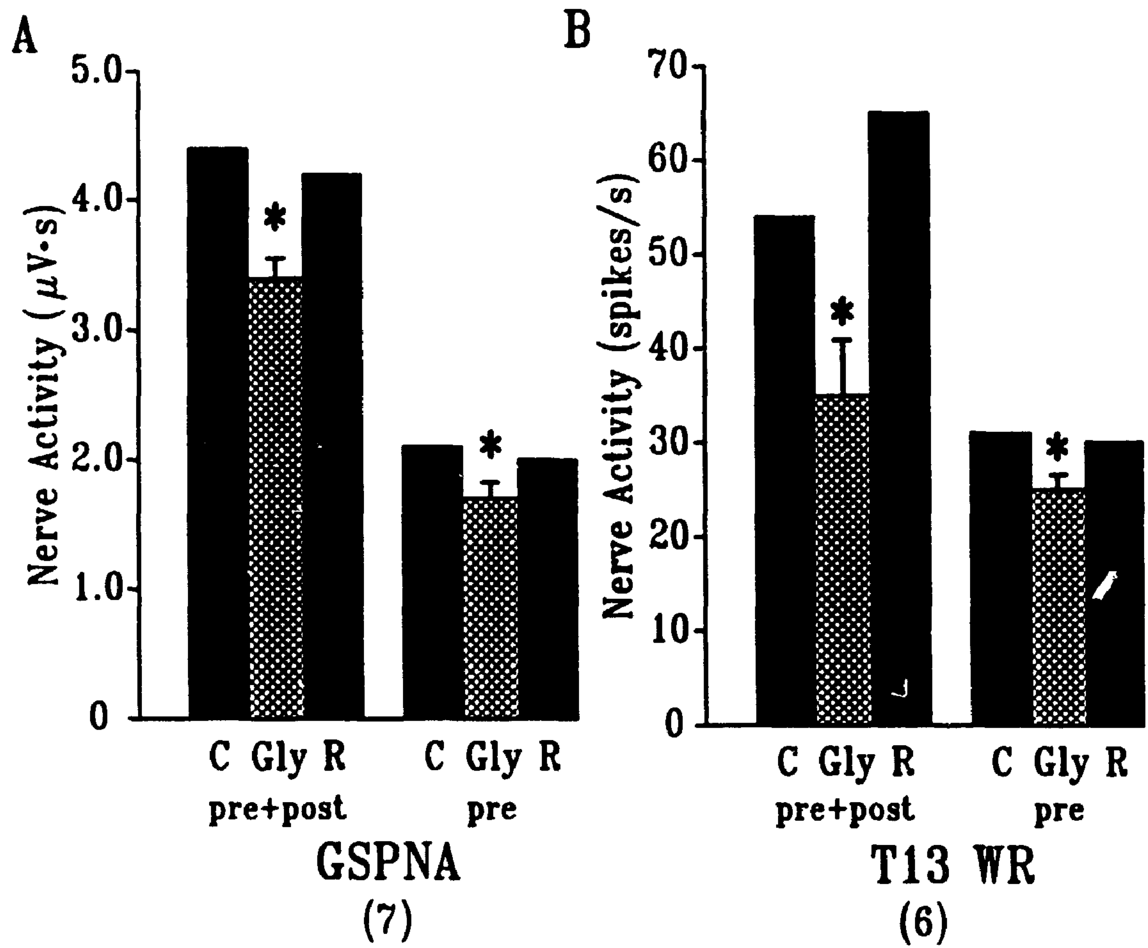


10
5
0
-5
-10
-15
-20
-25
-30
-35
-40
-45
Change (%)
*
*
*
*
RNA
SNA
MNA
T13WR
GSPNA

glycine was injected into the same site in the RVLM before and after ganglionic blockade to compare preganglionic responses to those of the mixed nerve. Before this protocol could be used it was necessary to determine if the same sympathetic and arterial pressure responses could be elicited by two consecutive glycine injections into the same site. In initial experiments, a glycine injection delivered sooner than 65 min after the first injection appeared to produce a smaller response. Therefore effects of 2 injections separated by an interval of at least 65 min were tested in 6 sites, in 5 rats. The first glycine injection produced a fall in arterial pressure of 28 ±4% (from 107 to 78 mmHg; pooled S.E.=3.1) and a decrease in sympathetic (greater splanchnic or renal) nerve activity of 21 ±5% (from 3.6 to 2.7 $\mu V \cdot s$; pooled S.E.=0.27). A second injection of glycine into the same site decreased arterial pressure 32 ±4% (from 95 to 64 mmHg; pooled S.E.=3.9) and sympathetic activity by 24 ±2% (from 3.5 to 2.7 $\mu V \cdot s$; pooled S.E.=0.19). The first and second responses to glycine were not statistically different. For this reason in subsequent experiments, the second glycine injection was not made until at least 65 min after the first injection.

Before ganglionic blockade, 7 glycine injections in 7 rats decreased greater splanchnic discharge by 28 ±4% and 6 injections in 6 rats decreased white ramus activity by 43 ±9% (Fig. 6). After ganglionic blockade glycine injections into these same brainstem sites decreased preganglionic greater splanchnic discharge by 18 ±4% and decreased preganglionic white ramus activity by 34 ±8% (Figs. 4-6). The responses of the preganglionic fibres in each nerve were significantly smaller than the responses of nerve bundles that contained postganglionic components (Fig. 6).

Figure 6. Mean responses of greater splanchnic nerves (A; n =7) and 13th thoracic white rami (B; n =6) to unilateral injections of glycine before (nerve bundle contains both pre- and postganglionic fibres; pre + post) and after (nerve bundle contains preganglionic fibres only; pre) ganglionic blockade. The solid bars are average discharge during 60 s control (C) and recovery (R) periods. The cross-hatched bars are discharge during the 10 s of maximum response to glycine (Gly). Variability is indicated by the pooled standard error and asterisks indicate significant difference from control. Glycine injection caused a significantly greater reduction in nerve activity before ganglionic blockade than after ganglionic blockade.


A
5.0
4.0
3.0
2.0
1.0
0
Nerve Activity (μV·s)
*
*
C Gly R
pre+post
C Gly R
pre
GSPNA
(7)
B
70
60
50
40
30
20
10
0
Nerve Activity (spikes/s)
*
*
C Gly R
pre+post
C Gly R
pre
T13 WR
(6)

The decrease in preganglionic white ramus activity was significantly greater than the decrease in preganglionic greater splanchnic nerve activity (Fig. 5).

1.4.3 Simultaneous recordings from renal and splenic nerves.

Four injections of glycine into the RVLM were made in 4 rats in which neural activity was recorded from renal and splenic nerves simultaneously. Glycine injection reduced renal nerve discharge by 42 ±2% (from 2.1 ±0.4 to 1.2 ±0.2 μV·s; pooled S.E.=0.11) and splenic nerve discharge by 32 ±2% (from 2.7 ±0.1 to 1.8 ±0.1 μV·s; pooled S.E.=0.11). Although, in 3 animals, blockade of the RVLM produced a greater reduction in renal nerve activity than splenic nerve activity, in 1 animal, RVLM blockade produced a similar response in both nerves.

1.4.4 Control injections.

In 3 rats, 3 control injections (180 nl) of sodium chloride (1.0 M) into the RVLM increased arterial pressure by 7 ±3% and pre- and postganglionic greater splanchnic nerve activity by 8 ±1%. The same volume of glycine (1.0 M) microinjected into the same sites reduced arterial pressure by 27 ±3% and greater splanchnic nerve activity by 16 ±4%. The saline injections were the first injections made at each site.

1.4.5 Sympathetic responses to RVLM blockade in rats anaesthetized with alpha-chloralose.

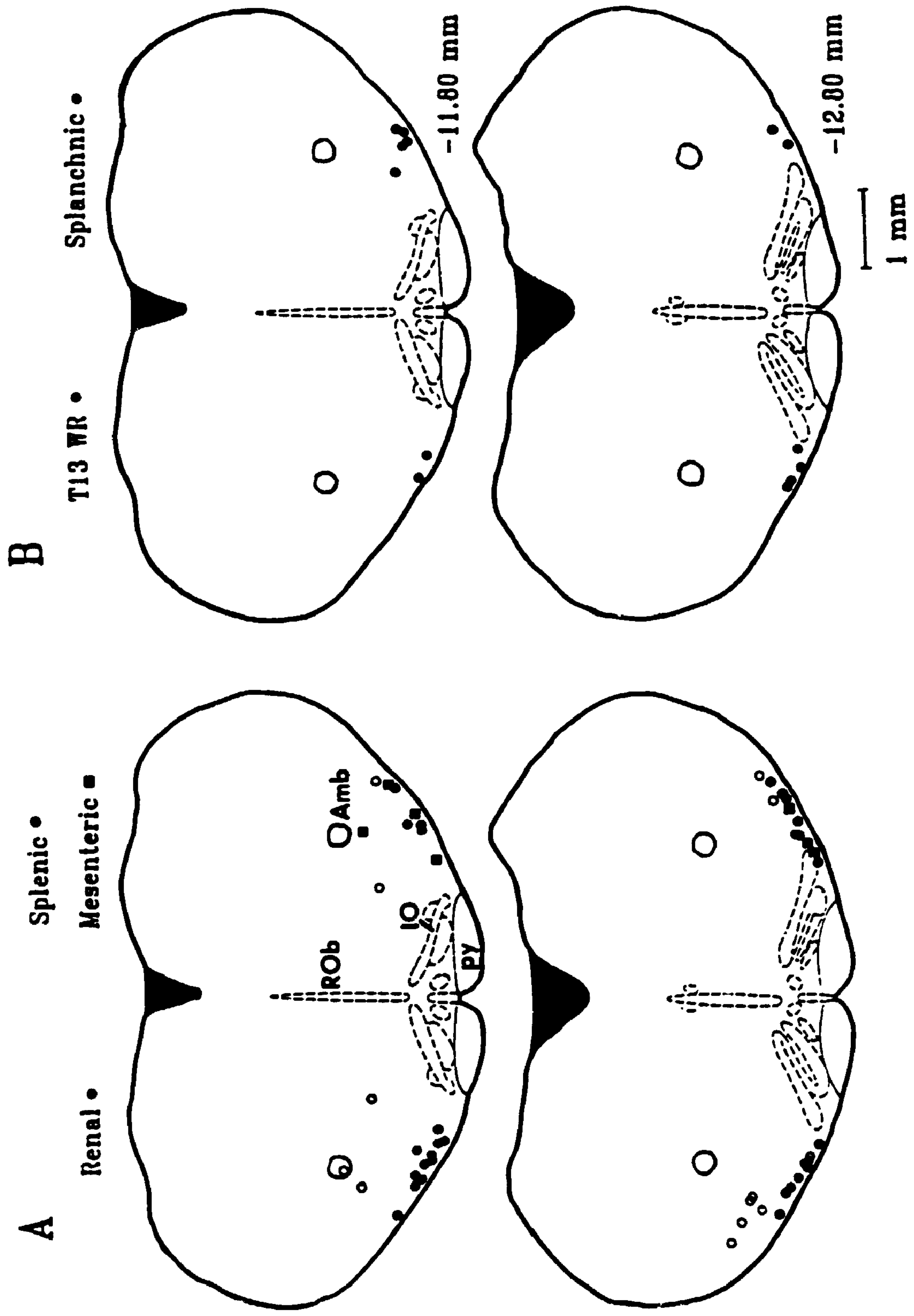
A previous study had shown that, in rats anaesthetized with alpha-chloralose, inhibition of activity of RVLM neurons increases the discharge of renal nerves (Poree and Schramm, 1989). For this reason, four unilateral injections of glycine were made into the RVLM of 3 rats anaesthetized with alpha-chloralose. However, glycine

injections decreased arterial pressure by 45 ±7% (from 121 to 67 mmHg; pooled S.E.=5.4) and renal nerve activity by 60 ±7% (from 2.3 to 0.9 μV·s; pooled S.E.=0.25). Although these responses to glycine tended to be greater than those observed in rats anaesthetized with urethane, statistically significant differences were not detected. In 2 of these animals, sharp, transient increases in arterial pressure preceded the large depressor response produced by glycine. These initial pressor responses following glycine injection have also been observed after bilateral glycine injection into the RVLM of cats anaesthetized with alpha-chloralose (Yardley *et al.*, 1989a).

1.4.6 Histological verification of injection sites.

The locations of the histologically verified injection sites in the RVLM, plotted on drawings (modified from Paxinos & Watson, 1986) of transverse sections of the rat medulla are shown in Fig. 7. Effective sites in which glycine produced changes in mean arterial pressure were localized in a region extending caudally from the most posterior extent of the facial nucleus to the rostral one-third of the inferior olive, lateral to the inferior olive and within 500 μm of the ventral surface. Sites in which glycine did not elicit an arterial pressure response are also plotted and most of these sites were found outside of this restricted area.

Figure 7. Drawings of transverse sections of rat medulla 11.80 and 12.80 mm caudal to bregma. Filled symbols represent injection sites in which glycine produced changes in arterial pressure. Open circles represent injection sites in which glycine did not elicit a change in arterial pressure. A: injection sites where renal (plotted on the left), splenic and mesenteric (plotted on the right) nerve activity was recorded. B: injection sites where preganglionic T_{13} white ramus (plotted on the left) and greater splanchnic (plotted on the right) nerve activity was recorded. Amb (nucleus ambiguus); ROb (raphe obscurus); IO (inferior olive); py (pyramidal tract).


A
Renal •
Splenic •
Mesenteric ■
ROb
IO
Amb
PY
B
T13 WR •
Splanchnic •
-11.80 mm
-12.80 mm
1 mm

1.5 Discussion

These experiments have shown that in rats, the sympathetic outflow from the RVLM is non-uniform to different visceral organs. Blockade of tonic activity in this area produced larger decreases in renal nerve activity than in splenic nerve activity, but failed to inhibit mesenteric nerve discharge consistently. This pattern of responses is similar to that which occurs after RVLM blockade in cats (Stein *et al.*, 1989; Yardley *et al.*, 1989a).

The decrease in renal sympathetic nerve activity observed after blockade of the RVLM in rats is consistent with changes in nerve activity previously reported in other species after similar RVLM blockade (Pilowsky *et al.*, 1985; Stein *et al.*, 1989; Yardley *et al.*, 1989a). Reductions in renal nerve activity have also been reported in rats following bilateral microinjections of lidocaine (Barrés, Lewis, Grosskreutz, Varner & Brody, 1989) or tetrodotoxin (Granata *et al.*, 1986) into the RVLM. These results are in conflict with those reported by Poree and Schramm (1989) in which bilateral injections of muscimol into the RVLM of rats anaesthetized with alpha-chloralose failed to decrease renal nerve activity. Since the rats in our study and those of Granata *et al.* (1986) and Barrés *et al.* (1989) were anaesthetized with urethane, it was possible that the differences in the renal nerve response were related to the different anaesthetics used. It has been reported that the hypotensive effects of bilateral electrolytic lesions of the RVLM are dependent on the type of anaesthetic and that persistent hypotension occurs particularly in animals anaesthetized with urethane (Cochrane *et al.*, 1988). However, as large decreases in renal nerve discharge were

still produced in chloralose-anaesthetized rats in our study, the conflicting finding of Poree and Schramm (1989) must relate to a factor other than the type of anaesthetic used in their study.

Changes in splenic nerve discharge after RVLM blockade may have been smaller than changes in renal nerve discharge because renal nerves contain more fibres that have vasomotor functions than do splenic nerves. However, the sympathetic innervation of the spleen is important in the control of blood pressure. In addition to arterioles and veins, splenic fibres innervate the capsule of the spleen. Contraction and relaxation of the splenic capsule occurs in response to unloading and loading of baroreceptors in cats and dogs (Brender & Webb-Peploe, 1969; Maass-Moreno & Rothe, 1991) and changes in splenic capsular tone play a crucial role in the capacitance of this vascular bed (Carneiro & Donald, 1977). Moreover, in single fibre studies, splenic and renal neurons all had vasomotor-like characteristics as they all responded to pressoreceptor stimulation and unloading (Meckler & Weaver, 1988). Therefore, differences in the function of individual neurons is not a likely explanation for different control by the RVLM. Alternatively, the renal nerve may contain a subpopulation of vasomotor fibres that are extremely sensitive to RVLM blockade and a group of fibres, like those of the splenic nerve, that are less sensitive to RVLM blockade. Since we recorded from nerve bundles containing many fibres, we have no data to support or refute this hypothesis. However, in a previous study, half of the single renal nerve fibres tested ceased firing after disruption of bulbospinal input whereas most of the splenic fibres continued to fire (Meckler & Weaver, 1988). This

finding in cats is clear evidence for differences in sensitivity among renal neurons to supraspinal sources of excitation.

The most unusual response to RVLM blockade was that of the mesenteric nerve. The lack of inhibitory responses of most mesenteric nerves to RVLM blockade suggests that ongoing discharge of these nerves originates from regions outside the RVLM. However, responses of mesenteric nerves are not easily interpretable, as they contain fibre groups which innervate blood vessels, myenteric ganglia and the intestinal mucosa (Costa & Furness, 1984), groups which may have unique responses to a given stimulus. Single fibre studies have revealed a population of mesenteric fibres having discharge which is not correlated with the arterial pulse pressure and which is insensitive to or excited by increases in arterial pressure (Stein & Weaver, 1988). Since arterial baroreceptors affect intestinal blood flow (Brooksby & Donald, 1971; Greenway & Lister, 1974) but only weakly affect intestinal motility (McAllen, 1986), these pressoreceptor-insensitive fibres may regulate motility and secretion. In the present study, the multifibre response of the mesenteric nerve to blockade of the RVLM probably involved excitatory and inhibitory responses among the subpopulations of mesenteric fibres. Since both baroreceptor activation and blockade of the RVLM decrease discharge of some neurons within the RVLM (Brown & Guyenet, 1985), it is conceivable that those postganglionic fibres which are excited by increases in arterial pressure are also excited by RVLM blockade, and are responsible for the increases in mesenteric discharge we observed in some nerve bundles. It is interesting that, despite the fact that 5 injections of glycine increased mesenteric

activity, all nerve bundles had a proportion of fibres that were baroreceptor-sensitive. Perhaps even the vasomotor fibres of the mesenteric nerve were excited by RVLM blockade.

The unequal effects of RVLM blockade on the discharge of renal, splenic and mesenteric sympathetic outflows are reflected in their preganglionic inputs. Blockade of RVLM neurons caused larger decreases in the discharge of preganglionic T_{13} white rami than in that of greater splanchnic nerves. This suggests the presence of different influences on two discrete populations of lower thoracic preganglionic neurons. Moreover, recent anatomical and electrophysiological studies suggest that rat renal postganglionic neurons receive most of their preganglionic input from T_{10} to L_1 white rami and that splenic and mesenteric neurons are innervated primarily by the greater splanchnic nerve (Ferguson, Ryan & Bell, 1986; Sripairojthikoon & Wyss, 1987; Chevendra & Weaver, 1991; Taylor & Weaver, 1992a). This indicates that the lower thoracic white rami and renal nerves are controlled by a central sympathetic pathway more dependent upon excitatory drive from the RVLM than are the pathways to the spleen and intestine.

Blockade of the RVLM did not decrease activity in preganglionic nerves to the same degree as it decreased the activity of the postganglionic nerves they innervate. How does postganglionic discharge decrease, to the extent it does, without a similar decrease in preganglionic discharge? Sympathetic ganglia act as integrative centres due mainly to the convergence and divergence of the preganglionic fibres on the ganglion cells (Polosa *et al.*, 1979). Convergence of several different preganglionic

fibres onto ganglion cells has been reported in the rat (Perri, Sacchi & Casella, 1970) as well as in other species (Wallis & North, 1978; Purves & Hume, 1981). In addition, anatomical studies show that there is considerable branching of individual preganglionic axons to innervate from 50 to 200 ganglion cells (Purves & Wigston, 1983). Since many preganglionic inputs are subthreshold, several inputs must sum to generate an action potential in the ganglion cell (McLachlan, 1974; Skok & Ivanov, 1983). Therefore, a loss of tonic activity (by blockade of the RVLM) in a small number of preganglionic axons could cause the loss of activity in many postganglionic neurons. Loss of rhythmicity in preganglionic firing after elimination of supraspinal input (Qu *et al.*, 1988) also could decrease summation of postsynaptic potentials leading to greater decreases in postganglionic firing.

Selective effects on different sympathetic nerves can occur because of topographical organization within the medulla. Studies on the functional organization of the RVLM in the cat have shown that neurons responsible for generating renal vasoconstriction were found mostly in the rostral portion of the RVLM, whereas those causing mesenteric vasoconstriction were found within the caudal portion (Lovick, 1987). Dampney and McAllen (1988) have shown topography in the medial/lateral axis in the caudal end of the RVLM of the cat. Muscle vasoconstrictors were more affected by lateral RVLM neurons and skin vasoconstrictors were more affected by medial neurons. Injections of lidocaine into the RVLM of the rat caused greater inhibition of lumbar chain and splanchnic nerves than injections into the rostral ventromedial medulla (Varner, Grosskreutz, Cox & Brody, 1989). However tonically

active RVLM neurons are not organized viscerotopically with respect to the kidney and spleen in the rat. Small (15 nl) microinjections of muscimol into sites distributed throughout the RVLM caused larger responses in renal nerves than in splenic nerves (Beluli & Weaver, 1991b). Although, viscertopy within the ventrolateral medulla may be organized according to the type (i.e visceral, skeletal muscle or cutaneous) rather than the body position of the vascular bed (McAllen & Dampney, 1990), topographic organization does not appear to be a likely explanation for differential responses of visceral nerves to RVLM blockade in the rat.

How might the brainstem selectively control different sympathetic outflows? Excitatory input from the RVLM to sympathetic preganglionic neurons probably is balanced by tonic sympathoinhibitory influences from other areas in the brain. Blockade of excitatory drive from the RVLM may leave an inhibitory system intact which contributes to the decrease in nerve activity. A difference in the balance of excitatory and inhibitory influences on different sympathetic nerves may explain the differential sympathetic responses to RVLM blockade. This inhibitory system may be responsible for the greater decreases in discharge of postganglionic renal nerves and preganglionic white rami and would suggest a tonic sympathoinhibitory system selective for this sympathetic pathway. Indeed, Taylor and Schramm (1987; 1988) reported that renal and gastrosplenic nerve activity increases after cervical spinal cord transection suggesting the presence of tonic inhibition of some spinal sympathetic systems in the intact rat.

The sympathetic nervous system is capable of producing distinct patterns of nerve

discharge to control selectively the functions of various visceral organs. For example, chemical or mechanical stimulation of visceral receptors often causes non-uniform excitation in sympathetic nerves innervating different organs (Calaresu, Tobey, Heidemann & Weaver, 1984; Meckler & Weaver, 1988; Stein & Weaver, 1988) and the largest output often is directed to the organ from which the reflex originates. Cardiovascular pressoreceptor stimulation also causes unequal responses in mesenteric, splenic and renal nerves (Meckler & Weaver, 1988; Stein & Weaver, 1988), a response pattern which also was observed in this study. Our findings indicate that tonic medullary influences, as well as reflex regulation of sympathetic discharge can be selective. When all influences are considered in combination, the central nervous system has a powerful mechanism by which it can exert specific sympathetic control over different visceral organs.

Chapter 2: Pre- and postganglionic sympathetic activity in white rami of rats.

Neuroscience Letters 115: 55-61, 1990.

2.1 Introduction

Preganglionic sympathetic fibres are often mixed with postganglionic fibres in peripheral nerves. The lumbar and greater splanchnic nerves of the cat (De Groat & Lalley, 1974; Kuo, Yang, Yamasaki & Krauthamer, 1982; Baron, Janig & McLachlan, 1985) and the greater splanchnic nerve of the rat (Celler & Schramm, 1981) have been shown to contain both pre- and postganglionic axons and some anatomical illustrations show ganglia in the splanchnic nerve located between its emergence from the thorax and its termination in the coeliac ganglion (Asfoury, 1971; Baljet & Drukker, 1979). Sympathetic ganglia known as intermediate or satellite ganglia are also frequently found in the preganglionic connections to the cervical portion of the sympathetic chain (Foley, 1945; Boyd, 1957). However, information about the composition of the communicating rami to the thoracolumbar sympathetic chain is limited.

In the previous investigation of brainstem influences on preganglionic and postganglionic nerves that provide innervation to the abdominal viscera, an empirical observation was made that these communicating rami contained postganglionic discharge that could not be attributed to the gray rami fibres. In this study evidence of this postganglionic discharge as well as the results of a histological study conducted to determine if the cell bodies of these postganglionic fibres are contained within the communicating rami themselves will be presented.

2.2 Methods and Results

2.2.1 *Electrophysiological Experiments.*

Male Wistar rats (200-400 g; Charles River, Canada) were anaesthetized with urethane (1.4 g/kg, i.p.), a tracheal tube was inserted, and the jugular vein and the femoral artery was cannulated. Arterial blood samples were withdrawn periodically for analysis of pH, pO_2 and pCO_2 and deviations from normal were corrected. Body temperature was maintained at 37°C. Physiological saline was infused (0.6 ml/hr) throughout the experiment to compensate for fluid loss. The animals were artificially respired and paralysed with gallamine triethiodide (20 mg/kg initially, followed by doses of 10 mg/kg as needed).

The anatomy of the thoracolumbar chain region as observed in these experiments is shown in Fig. 1. As described in the previous study, the left sympathetic chain was exposed by a flank incision and the greater splanchnic nerve was identified and followed rostrally to its junction with the sympathetic chain. The diaphragm was detached from the chest wall to permit adequate exposure of the 13th thoracic (T_{13}) ganglion. The rostral and caudal T_{13} white rami were identified and one of the two was selected randomly, dissected free from surrounding tissue and its distal end was either severed or crushed (Fig. 1). The central end was placed on stainless steel bipolar electrodes for recording multifibre electrical activity and was covered with dental impression medium (Regasil, Dentsply International Inc., Milford, DE). The amplified neural signals were monitored on an oscilloscope and recorded on magnetic tape. Arterial pressure and heart rate were displayed on a Grass Instruments

polygraph.

In one group of animals a sample of neural activity was recorded for 2 min before and for 2 min after crushing the distal end of the ramus. In another group of animals neural activity was recorded from the central ends of severed rami for 2 min before a bolus of the ganglionic blocking drug, chlorisondamine (5 mg/kg,i.v., Ciba-Geigy Canada Ltd.), was injected. In order to maintain arterial pressure at levels comparable to those before ganglionic blockade, phenylephrine was infused for the duration of the experiment. Once arterial pressure, heart rate and neural discharge had stabilized, another 2 min of activity was recorded. At the end of the experiments, an overdose of urethane was given and background electrical noise was recorded for 2 min.

Neural activity of intact or crushed nerves was recorded on magnetic tape, digitized, rectified and integrated cumulatively during 10 s periods. Integrated background electrical noise was subtracted from the total integrated signal to quantify only the neural signal. Activity of severed nerves before and after injection of chlorisondamine was quantified by voltage integration and in counts of spikes/s after window discrimination. Both methods of quantitation were used because after ganglionic blockade these nerve bundles had only a few active fibres firing and marked decreases in integrated voltage were observed. Integrated values of neural activity are greatly affected by the amplitudes of single spikes in few-fibre preparations and may have underestimated the activity remaining following ganglionic blockade. Spike counting allowed action potentials of relatively low voltage to be detected and weighted equally with larger single action potentials. The thresholds for

window discrimination were set close to the maximum amplitude of background electrical noise during meticulous inspection of nerve activity monitored on an oscilloscope and by an audio monitor to exclude background noise and to include as much nerve activity as possible in the quantitation procedures.

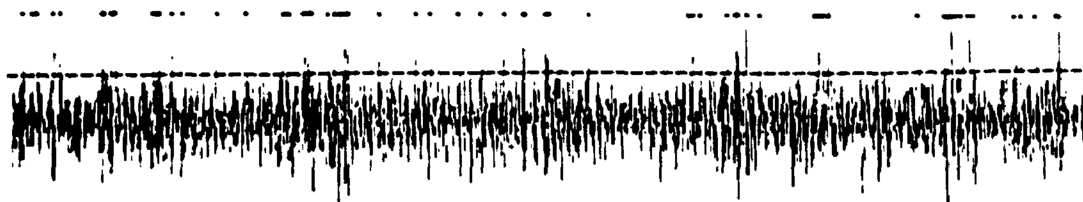
A paired t-test was used to determine statistical significance of changes in neural discharge after ganglionic blockade with chlorisondamine. Differences were considered significant when $p<0.05$. Data are expressed as means ± S.E.M.

Spontaneous activity recorded in the intact communicating rami of 5 rats was reduced 65 ±9% by crushing the distal ends. Activity before crushing represents the total discharge of the communicating bundle including visceral afferent, gray ramus efferent and white ramus efferent activity. Neural activity after crushing represents the proportion of this total activity that is central in origin (i.e. via the white ramus fibres).

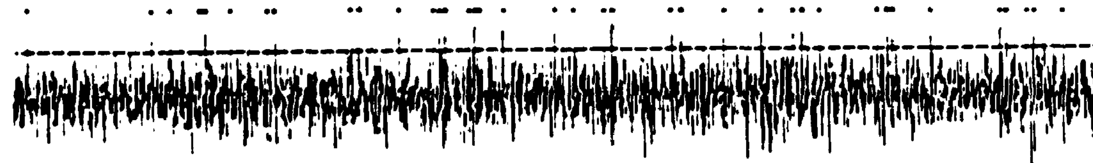
Ongoing activity of white ramus fibres (in cut nerve preparations) was recorded before and after ganglionic blockade. As illustrated in Fig. 8, the discharge of this nerve was significantly reduced following ganglionic blockade. Table 2 gives the responses of 7 rats to i.v. administration of chlorisondamine. The mean decrease in integrated nerve activity as a result of ganglionic blockade in these animals was 47 ±9% ($t = 2.83$) and the decrease in the same activity when spike-counted was 49 ±10% ($t = 2.90$). The mean changes in nerve activity after ganglionic blockade quantified by either method were similar. The magnitude of the neural response to ganglionic blockade or crushing the nerve was independent of the ramus bundle (rostral or caudal) selected for recording.

Figure 8. Neurograms of sympathetic discharge from the T_{13} connecting ramus in rat no. 890419 during a control period and following ganglionic blockade with chorisondamine. Background noise is shown in the third panel. Vertical calibration is 20 μV. The interrupted lines indicate the thresh·old voltage set to trigger the window discriminator and the dots indicate spikes of discharge counted. Discharge of this nerve was decreased substantially after ganglionic blockade.

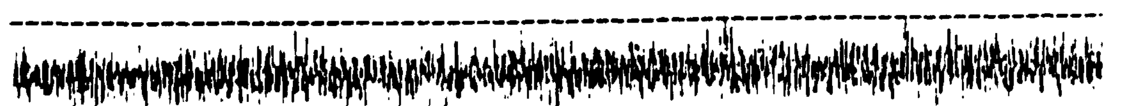
CONTROL



GANGLIONIC BLOCKADE



NOISE



1 s

Table 2. Effect of ganglionic blockade with chlorisondamine on electrical activity of T_{13} white rami.

Animal Number	**Control** $\mu V \cdot s/10s$	spikes/s	**Blockade** $\mu V \cdot s/10s$	spikes/s	**Decrease**	
890323	9.5	117.4	5.9	66.2	38%	44%
890328	7.9	47.2	1.6	1.9	80%	96%
890404	4.8	53.6	4.0	42.6	17%	21%
890410	2.3	3.9	1.6	1.2	30%	70%
890418	5.5	22.9	3.7	15.4	33%	33%
890419	2.3	16.0	1.1	8.6	52%	46%
890426	11.1	72.7	2.5	46.7	77%	35%
Mean ±S.E.M.	6.2 ±1.3	48 ±15	2.9 ±0.7*	26 ±10†	47 ±9%	49 ±10%

* significantly different from control (t =2.83)
† significantly different from control (t =2.90)

2.2.2 ***Histological Observations.***

The communicating rami of the 10th thoracic to the 1st lumbar sympathetic ganglia were dissected from rats perfused transcardially with 300 ml of physiological saline followed by 300 ml of a fixative solution containing 2% paraformaldehyde in a solution of 15% saturated picric acid in phosphate buffer, pH 7.4 (Zamboni's fixative). The tissue was placed in Zamboni's fixative for 30 minutes before it was transferred to 20% sucrose in phosphate buffered saline and stored at 4°C. Frozen tissue was cut at 8-10 μm at -20°C and directly mounted onto gelatinized slides. The sections were stained with thionin to visualize cell bodies and were viewed using a Leitz microscope. Numbers of cells were counted and corrected for double counting using Abercrombie's equation (Abercrombie, 1946).

We searched for neurons in the white rami between their exit from the spinal nerves to their entry into the 13th thoracic ganglion. Thionin-stained neurons were observed within 4 communicating ramus nerve bundles (3 rats). The somas were large (average diameter of 30μm), and oval with large nuclei. Typically, one or two nucleoli could also be detected (Fig. 9). In 2 nerve bundles, single thionin-stained isolated cell bodies were found. Two other bundles each contained a cluster of neurons. One cluster contained 10 cells, the other 19 cells. In 7 rami (5 rats) no cell bodies were found along the course of this nerve. The communicating rami were removed from one animal in which fluorescent dye (Fast Blue, Sigma Chemicals; 4% Fluoro Gold, Flurochrome Inc.) had been applied to crushed renal and splenic postganglionic nerves. Nine fluorescent-labelled postganglionic neurons were located

Figure 9. Photomicrographs of (A) a single thionin-stained neuron with two nucleoli and (B) a ganglion-like cluster of thionin-stained neurons within a T_{13} connecting ramus bundle. Bar = 15 μm.

A

B

in a cluster within the ramus nerve bundle. This result suggests that the thionin-stained cell bodies we observed were also postganglionic.

2.3 Conclusions and discussion.

Ganglionic blockade with chlorisondamine demonstrated the presence of neural discharge that must have been due to both pre-and postganglionic fibres in the 13th thoracic communicating ramus of the rat. In our search for the postganglionic cell bodies of these fibres we observed neurons along the course of only 4 of 7 rami. Indeed, Celler and Schramm (1981) found very few cell bodies within the trunk of the greater splanchnic nerve and even the 60 to 100 postganglionic renal neurons found by Sripairojthikoon and Wyss (1987) probably cannot account for all the postganglionic activity which can be recorded in a rat splanchnic nerve (Celler & Schramm, 1981). Celler concluded that the majority of the postganglionic axons in this nerve originate in the thoracic sympathetic ganglia (Celler & Schramm, 1981). Some of the cell bodies contained in the ramus bundle may be ganglioblasts that have halted prematurely in the process of their migration from the neural crest to form paravertebral ganglia during development of the sympathetic nervous system (Yip, 1986). However, blockade of the discharge of a few aberrant cells could not be responsible for the large decrease in ongoing nerve activity caused by chlorisondamine. Since the electrophysiological responses were so consistent, the population of neurons causing these responses must occur more regularly than we were able to detect in our histological examinations.

Most of the cell bodies within the rami were located in clusters that may be part of intermediate ganglia. Intermediate ganglia have been found in the cervical sympathetic trunk (Foley, 1945) and in the lumbar splanchnic nerves of the cat (Baron *et al.*, 1985). Boyd (1957) described two principal groups of intermediate ganglia in the human, a cranial group of ganglia lying in the cervical and uppermost thoracic segments and a caudal group related to the lumbar and lowermost thoracic segments. Most of these ganglia are located in the course of the rami communicantes. However, Boyd (1957) also found similar ganglia in or near the ventral roots of the spinal cord, close to the dorsal root ganglion and within the spinal nerves themselves (Boyd, 1957). In our study, intermediate ganglia located in the spinal nerves or the ventral roots may explain the large and consistent decreases in nerve activity in the face of the small number of postganglionic cell bodies found.

It is important to take into account the presence of these intermediate ganglia in anatomical studies which count the number of axons in a preganglionic nerve bundle or which predict the composition of a nerve by degeneration experiments. For example, ventral root rhizotomy will eliminate preganglionic axons but may also cause the degeneration of postganglionic axons that originate in an a ganglion located within the ventral root (Brooks-Fournier & Coggeshall, 1981; Kuo *et al.*, 1982). Discharge from these postganglionic fibres must also be considered in electrophysiological experiments involving stimulation of or recording from preganglionic nerve bundles. The responses of preganglionic fibres may differ from those of postganglionic fibres contained within the same nerve bundle (Morrison & Whitehorn, 1984; Hayes &

Weaver, 1990). Finally, the presence of intermediate ganglia is relevant in denervation experiments since simply removing the sympathetic chain in rats would not provide complete chronic sympathetic denervation (Boyd, 1957).

Chapter 3: Evidence for descending tonic inhibition specifically affecting sympathetic pathways to the kidney in rats.

Journal of Physiology (London) 434: 295-306, 1991.

3.1 Introduction

In recent years, numerous studies have explored the properties of sympathoexcitatory neurons located in the rostral ventrolateral medulla (RVLM) and their role in maintaining tonic vasomotor tone. However, the understanding of inhibitory influences within the sympathetic nervous system is much less complete than that of excitatory pathways.

3.1.1 Tonic inhibition of sympathoexcitatory neurons in the RVLM

Excitatory drive from RVLM neurons is regulated by tonic sympathoinhibitory influences from other areas in the brainstem such as the caudal ventrolateral medulla (CVLM) or medullary raphe nuclei.

The caudal ventrolateral medulla

Vasodepressor neurons in the caudal medulla were first observed in the experiments of Feldberg and Guertzenstein (1972; 1976), Guertzenstein (1973) and Guertzenstein and Silver (1974) who showed that topical application of nicotine to the ventral surface of the cat medulla caused large decreases in arterial blood pressure. The location of this depressor area is centred around a group of noradrenergic cells (A1 cell group; Dahlstrom & Fuxe, 1964) although neurons containing other neurotransmitters and neuropeptides are also found in this region (Hokfelt, Lundberg,

Tatemoto, Mutt, Terenius, Polak, Bloom, Sasek, Elde & Goldstein, 1983; Chronwall *et al.*, 1985; Mugnaini & Oertel, 1985; Melander, Hokfelt, Rokaeus, Cuello, Oertel, Verhofstad and Goldstein, 1986; Kihara & Kubo, 1989). Pharmacological studies by Granata, Kumada and Reis (1985b) and Granata *et al.* (1986) have suggested that A1 neurons tonically inhibit sympathetic activity. However, more recent evidence has indicated that vasodepressor neurons in the CVLM utilize gamma-aminobutyric acid (GABA) as the inhibitory neurotransmitter (Blessing, 1988; Blessing & Li, 1989).

Electrical stimulation of neurons in the CVLM or local microinjection of L-glutamate reduces arterial blood pressure, sympathetic nerve activity and resistance in mesenteric, renal and hindquarter vascular beds (Blessing & Reis, 1982; Willette, Krieger, Barcas & Sapru, 1983a; Willette, Barcas, Krieger & Sapru, 1983b; Blessing, Sved & Reis, 1984; Willette, Punnen, Krieger & Sapru, 1984a; Pilowsky *et al.*, 1985; Sved, Blessing & Reis, 1985; Willette *et al.*, 1987; Blessing, 1988; Beluli & Weaver, 1991a). Conversely, inhibition of activity of neurons in this region by electrolytic lesions, or microinjections of kainic acid or inhibitory amino acids increases arterial pressure mediated by increases in sympathetic nerve discharge and regional vascular resistances (Blessing, West & Chalmers, 1981a; West, Blessing & Chalmers, 1981; Blessing *et al.*, 1984; Willette, Punnen, Krieger & Sapru, 1984b; Granata *et al.*, 1985b; 1986; Pilowsky *et al.*, 1985; Sved *et al.*, 1985; Willette *et al.*, 1987; Dampney, Blessing & Tan, 1988). In addition, activation of CVLM neurons caused differential changes in the activity of different sympathetic nerves (Dembowsky, Czachurski & Seller, 1989; Beluli & Weaver, 1991a). The existence of inhibitory projections

descending from the CVLM to the spinal cord is controversial (Fleetwood-Walker, Coote & Gilbey, 1983; Hudson, Fuxe, Goldstein & Kalia, 1986; Dembowsky *et al.*, 1989), but most evidence has suggested that the CVLM vasodepressor response depends on the integrity of sympathoexcitatory neurons located in the RVLM. Inhibition of RVLM neuronal activity with tetrodotoxin (Granata *et al.*, 1986) or muscimol (Willette *et al.*, 1984b) prevented the sympathoexcitatory effects produced by kainic acid or muscimol injection into the CVLM. Blockade of GABAergic receptors in the RVLM also eliminated arterial pressure responses to excitation or inhibition of CVLM neurons (Blessing, 1988; Gatti & Gillis, 1991). Anatomical tracing studies have shown that few CVLM neurons have axonal projections to sympathetic preganglionic neurons in the intermediolateral cell column (IML) of the spinal cord (Amendt, Czachurski, Dembowsky & Seller, 1979; Blessing, Goodchild, Dampney & Chalmers 1981b; Ross *et al.*, 1984). However, dense axonal projections from CVLM neurons to bulbospinal neurons located in the RVLM have been demonstrated (Blessing & Li, 1989; Van Bockstaele, Pieribone & Aston-Jones, 1989; Blessing, 1990; Li, Wesselingh & Blessing, 1992b). Furthermore, RVLM bulbospinal neurons were inhibited by chemical stimulation of the CVLM and were excited by inhibition of ongoing CVLM neuronal activity indicating that the CVLM-RVLM pathway is tonically active (Agarwal, Gelsema & Calaresu, 1989; Li, Gieroba, McAllen & Blessing, 1991). Activation of baroreceptors excited some CVLM neurons which were orthodromically excited by electrical stimulation of the nucleus tractus solitaris (NTS) and antidromically activated by stimulation in the RVLM (Agarwal &

Calaresu, 1991). Therefore, vasodepressor neurons in the caudal medulla act substantially by tonically inhibiting neurons in the rostral medulla which project to sympathetic preganglionic neurons in the spinal cord. Tonic sympathoinhibition from the CVLM is likely an integral part of the baroreceptor reflex pathway (Sun & Guyenet, 1985; Gordon, 1987; Agarwal, Gelsema & Calaresu, 1990; Masuda, Terui, Koshiya & Kumada, 1991).

The raphe nuclei

The raphe nuclei (pa. lus, obscurus and magnus) are contained within the medial depressor area first described by Ranson and Billingsley (1916) and Wang and Ranson (1939). Electrical stimulation or chemical activation of raphe cell bodies with L-glutamate usually elicits marked decreases in arterial pressure and sympathetic nerve activity (Cabot, Wild & Cohen, 1979; Gilbey, Coote, Macleod & Peterson, 1981; McCall & Humphrey, 1985; Minson, Chalmers, Caon & Renaud, 1987; McCall, 1988a). In addition, midline medullary lesions increase tonic sympathetic nerve discharge in both baroreceptor denervated cats and those with baroreceptor nerves intact (Barman & Gebber, 1978). These lesions failed to affect baroreceptor reflex mediated inhibition of sympathetic discharge (McCall & Harris, 1987). These observations suggest that neurons in the raphe nuclei provide tonic inhibition of sympathetic nerve activity that is independent of baroreceptor inputs. Electrophysiological studies have identified raphe neurons whose spontaneous activity is synchronized to the 2-6 Hz slow wave of sympathetic nerve discharge. The firing rate of these neurons increases during barorec ptor activation suggesting that they have

a sympathoinhibitory function (Morrison & Gebber, 1982; 1984; Barman & Gebber, 1989; McCall & Clement, 1989). Two studies have shown that neurons in the raphe nuclei provide differential control of different sympathetic nerves. Barman *et al.* (1984) have identified raphe neurons that have spontaneous activity more strongly correlated with renal than with inferior cardiac sympathetic nerve discharge. In addition, electrical stimulation of the raphe decreased renal sympathetic nerve activity but increased lumbar sympathetic nerve activity (Futuro-Neto & Coote, 1982).

Raphe neurons may influence sympathetic activity by tonically inhibiting sympathoexcitatory neurons in the RVLM. Anatomic work has shown that the RVLM receives projections from the caudal raphe nuclei (Bobillier, Seguin, Petitjean, Salvert, Touret & Jouvet, 1976; Van Bockstaele *et al.*, 1989). Electrical stimulation of the raphe nuclei inhibited spontaneous activity of sympathoexcitatory RVLM neurons (McCall, 1988a). Furthermore, many raphe sympathoinhibitory neurons can be antidromically activated by electrical stimulation in the region of the RVLM (Barman & Gebber, 1989). Since raphe inhibitory neurons fire earlier than RVLM sympathoexcitatory neurons relative to the peak of the cardiac-related slow wave in sympathetic nerve activity, raphe neurons may provide tonic inhibitory input to neurons in the RVLM (Gebber & Barman, 1988). The decreases in sympathetic activity caused by electrical stimulation of the raphe nuclei are mediated by GABA because this inhibition can be blocked by the GABA-receptor antagonists bicuculline and picrotoxin and is potentiated by diazepam (McCall & Humphrey, 1985). Microiontophoresis of bicuculline in the RVLM increases the tonic discharge of

sympathoexcitatory RVLM neurons and blocks the raphe-evoked inhibition of RVLM neuronal activity (McCall, 1988a). Since cell bodies immunoreactive for GABA have been found in the region of the RVLM (Ruggiero, Meeley, Anwar & Reis, 1985; Kihara & Kubo, 1989; Blessing, 1990), raphe-evoked inhibition of RVLM neurons is likely mediated by adjacent inhibitory GABA interneurons in the region of the RVLM.

3.1.2 Tonic bulbospinal inhibition of sympathetic activity

Tonic sympathetic excitation may also be balanced by inhibition descending in pathways from the brainstem to the spinal cord. Tonic inhibition of sympathetic nerves was first described by Alexander (1946) who demonstrated that ongoing discharge of cardiac nerves, which was completely abolished by transection of the brainstem at the level of the obex, could be partially restored by subsequent transection of the cervical spinal cord. Several more recent studies have revealed an inhibitory system that descends in the dorsolateral funiculus of the spinal cord. These axons are responsible for tonic inhibition of the spinal component of the somato-sympathetic reflex (Dembowsky, Czachurski, Amendt & Seller, 1980) and may also be the path of inhibition of purely visceral reflexes (Müller, Dembowsky, Czachurski & Seller, 1988). This inhibition is thought to originate in the medulla and is independent of baroreceptor inputs (Dembowsky *et al.*, 1980). Electrical stimulation of the dorsolateral funiculus causes a reduction in blood pressure and in sympathetic nerve activity after, but not before, spinal cord transection (Coote & Macleod, 1974; Schramm & Chornoboy, 1982; Schramm & Livingstone, 1987). One explanation for

the appearance of sympathoinhibition only after cord transection may be that this inhibitory system is maximally driven by supraspinal neurons or by neurons in the cervical spinal cord (Schramm & Livingstone, 1987). Therefore, stimulation of the spinal pathway in the intact animal would have no effect on sympathetic discharge (Schramm & Livingstone, 1987). Although stimulation of this pathway after spinal cord transection reduces renal nerve activity by 50%, stimulation has little effect on lumbar sympathetic discharge (Taylor & Schramm 1988), suggesting that the inhibition is selective for certain sympathetic nerves. Schramm and Livingstone (1987) propose that this inhibitory system has the strongest effect on nerves capable of generating activity after spinal cord transection. Their hypothesis is supported by the fact that, in chloralose-anaesthetized rats, renal and gastrosplenic nerve activity increases after cord transection, whereas lumbar chain discharge decreases (Taylor & Schramm, 1987; 1988). In cats, blockade of the RVLM causes small but significant decreases in the discharge of mesenteric nerves; subsequent spinal cord transection returns this discharge to control values (Stein *et al.*, 1989). These data suggest that in cats, elimination of excitatory influences from the RVLM leaves a bulbospinal inhibitory system intact and that interruption of this inhibitory system by cord transection allows mesenteric sympathetic discharge originating from spinal neurons to increase. These studies provide evidence for tonic bulbospinal inhibitory influences on sympathetic nerves in rats and cats. The finding that IPSPs can rarely be recorded from sympathetic preganglionic neurons argues against the existence of a tonically active sympathoinhibitory projection synapsing on these cells (Dembowsky, Czachurski &

Seller, 1985). Such a projection may, however, synapse on excitatory local interneurons or afferent fibres antecedent to preganglionic neurons and produce inhibition by disfacilitation.

3.1.3 Supraspinal neurons responsible for tonic descending inhibition

The raphe nuclei

There is some evidence to support the role of a raphe-spinal sympathoinhibitory system. Anatomic studies show axonal projections from the midline raphe nuclei which descend, in part, in the dorsolateral funiculus to the IML of the spinal cord (Loewy, 1981; Loewy & McKellar, 1981). Discrete lesions placed in the dorsal part of the dorsolateral funiculus of the cervical spinal cord can abolish the inhibition of sympathetic activity produced by raphe stimulation (Coote & Macleod, 1975). In addition, Morrison and Gebber (1985) have shown that raphe sympathoinhibitory neurons with discharges correlated with sympathetic nerve activity can be antidromically activated by electrical stimulation of the IML and some raphe-spinal sympathoinhibitory neurons emit branches in the cervical spinal cord (Barman & Gebber, 1988). These observations are consistent with a sympathoinhibitory system that is integrated or relayed in the cervical spinal cord. Furthermore electrical stimulation of the medullary raphe completely inhibits sympathetic preganglionic neuronal discharge (Cabot *et al.*, 1979). The latency of this inhibitory effect is similar to the latency observed when raphe neurons are antidromically activated from the IML (Cabot *et al.*, 1979). Finally, a direct monosynaptic pathway between cells in the

raphe pallidus and caudal raphe magnus and adrenal sympathetic preganglionic neurons has been demonstrated using electron microscopy (Bacon, Zagon & Smith, 1990).

The A5 cell group

The A5 cell group is a collection of norepinephrine-containing cells located in the ventrolateral portion of the brainstem at the pontomedullary junction (Dahlstrom & Fuxe, 1964). Anatomical studies using radio-labelled amino acids (Loewy, McKellar & Saper, 1979a), horseradish peroxidase (Westlund, Bowker, Zeigler & Coulter, 1983), fluorescent dyes (Westlund *et al.*, 1983; Byrum, Stornetta & Guyenet, 1984) and neurotropic viruses (Strack *et al.*, 1989a; 1989b) have shown that A5 catecholaminergic cells project massively to the intermediolateral cell column (IML) of the spinal cord. In fact, up to 93% of all noradrenergic neurons in this area send an axon to the thoracic spinal cord and noradrenergic neurons comprise at least 90% of all spinally projecting neurons in this area (Byrum *et al.*, 1984). Electrophysiological experiments support the anatomical evidence. Spinally projecting neurons in the A5 region have been identified by antidromic activation of their axons in the spinal cord and their location correlates with the position of catecholamine-containing cells (Byrum *et al.*, 1984; Coote, 1985). Antidromic mapping demonstrates that the axons of these cells arborize mainly in the region of the IML (Huangfu, Koshiya & Guyenet, 1991).

Attempts to understand the role of A5 cells in the regulation of arterial pressure and sympathetic outflow have produced a confusing picture. Activation of cell bodies

in this region with injections of excitatory amino acids causes decreases in arterial pressure (Close, Neil & Loewy, 1982; Neil & Loewy, 1982; Stanek, Neil, Sawyer & Loewy, 1984) and decreases in the resistance of skeletal muscle and kidney vascular beds (Stanek *et al.*, 1984). The depressor responses to A5 stimulation were attenuated following injections of the catecholaminergic neurotoxin 6-hydroxydopamine (6-OHDA) into either the IML or the NTS (Loewy, Marson, Parkinson, Perry & Sawyer, 1986). Arterial pressure and vascular resistance responses to injections of glutamate into the A5 area were also prevented by pretreatment with intraventricular injections of 6-OHDA (Stanek *et al.*, 1984). These results indicate that A5-spinal projections have inhibitory influences on sympathetic nerve activity supporting arterial pressure. This idea is supported by iontophoretic studies which show that norepinephrine inhibits sympathetic preganglionic neurons via $alpha_2$-adrenergic receptors (Coote, Macleod, Fleetwood-Walker & Gilbey, 1981; Guyenet & Cabot, 1981; McCall, 1988b). Some evidence indicates that sympathoinhibition from the A5 region may not be tonically active since lesions involving A5 noradrenergic neurons do not change resting arterial pressure (Granata *et al.*, 1985a). However, Dormer and colleagues (1986) demonstrated that, whereas radio-frequency lesions produce a slight decrease in arterial pressure, lesions of cell bodies with kainic acid result in increased arterial pressure (Dormer, Andrezik, Person, Braggio & Foreman, 1986). The A5 region of the pons represents a relatively unexplored source of sympathoinhibition. The possibility that inhibition is mediated through A5-spinal pathways or that it is tonically active has yet to be resolved.

Recent electrophysiological experiments suggest that A5 cells have a sympathoexcitatory function. Most neurons in the A5 region with axons terminating in the IML of the spinal cord were inhibited by raising arterial pressure and by stimulation of the aortic depressor nerve. The spontaneous activity of some of these cells was correlated with splanchnic sympathetic nerve discharge and preceded the peak of sympathetic discharge by a latency similar to that of sympathoexcitatory neurons in the RVLM (Huangfu *et al.*, 1991). Furthermore, microinjection of the excitatory amino acid N-methyl-D-aspartate (NMDA) into the A5 region increased splanchnic and renal sympathetic nerve discharges but usually decreased lumbar sympathetic nerve activity. The responses of the splanchnic nerve to NMDA injection were reduced by neurotoxic lesion of noradrenergic cell bodies in the A5 area and by intrathecal administration of prazosin, an alpha$_1$-adrenergic receptor antagonist (Huangfu, Hwang, Riley & Guyenet, 1992) indicating that the excitatory responses are mediated by a different adrenergic receptor in the spinal cord than are inhibitory responses. The sympathetic responses were accompanied by small decreases in arterial pressure and heart rate (Huangfu *et al.*, 1992).

3.2 Rationale for the present study

Blockade of excitatory neurons in the RVLM may leave unbalanced a tonically active bulbospinal sympathoinhibitory system which affects discharge of postganglionic renal and splenic nerves and their preganglionic inputs, the 13th thoracic (T_{13}) white rami and the greater splanchnic nerves. This hypothesis was

tested by comparing the magnitudes of nerve discharge observed after spinal cord transection to those seen following bilateral blockade of excitatory influences from RVLM neurons. Effects of spinal cord transection on discharge of T_{13} white rami were compared to effects of unilateral RVLM blockade presented in an earlier study (Hayes & Weaver, 1990).

A second objective of this project was to locate the supraspinal neurons which drive this sympathoinhibitory system. The A5 region of the pons was explored with small microinjections of the inhibitory amino acid glycine to locate groups of tonically active neurons that have inhibitory effects on sympathetic nerve activity.

3.3 Methods

Preparation of animals.

Male Wistar rats (200-400 g; n=34; Charles River, Canada) were anaesthetized with urethane (1.4 g/kg,i.p., Aldrich Chemical Company, USA) and tracheal tubes were inserted. The left jugular vein and the right femoral artery were cannulated for delivery of solutions and drugs and for monitoring arterial blood pressure, respectively. Arterial blood samples (~100 μl) were withdrawn periodically for measurement of pH, pO_2 and pCO_2 (pH/blood gas analyser, model 170, Corning Medical, USA) and deviations from normal were corrected by i.v. administration of sodium bicarbonate or by adjusting respiratory rate and/or tidal volume. Body temperature was maintained at 37°C with a heating pad. Physiological saline was infused slowly at a rate of 0.6 ml/hr throughout the experiment to compensate for fluid loss. The animals were artificially respired with oxygen-enriched air and paralysed with gallamine triethiodide (20 mg/kg initially, followed by doses of 10 mg/kg as needed, Rhone-Poulenc, Canada). Before each supplemental dose of gallamine was given, the animal's plane of anaesthesia was assessed by examining palpebral and withdrawal reflexes. Additional urethane (0.1 g/kg, i.v.) was given if needed.

The rats were placed in a stereotaxic frame (David Kopf Instruments, USA). The left sympathetic chain, the anterior bundle of the right greater splanchnic nerve or postganglionic renal and splenic nerves were exposed via a retroperitoneal approach. To expose the T_{10}-L_1 white rami, the psoas muscle was retracted laterally by sutures placed around the muscle and the sympathetic chain was carefully isolated as

described previously (Hayes & Weaver, 1990; Hayes *et al.*, 1990a). Small bundles of all sympathetic nerves were identified, dissected free from surrounding tissue and severed. The central ends were placed on stainless-steel bipolar electrodes for recording multifibre electrical activity. Exposed nerves were covered with dental impression medium (Regasil, Dentsply International Inc., U.S.A.) to isolate the nerve and electrode from the surrounding tissue and to prevent dehydration. In all animals a pneumothorax was made to eliminate artifacts in the neural recordings caused by respiratory movements. Neural discharge was amplified with a Grass P511 amplifier (Grass Instrument Company, USA) at a bandwidth of 30 Hz to 3 kHz. The amplified signals were monitored on an oscilloscope, recorded on magnetic tape (Racal, model 7DS, UK) and the integrated neural signals were displayed along with arterial pressure and heart rate on a Grass Instruments polygraph. In 3 experiments, discharge of renal and splenic nerves was recorded simultaneously.

To ensure that recordings were from sympathetic nerves containing vasomotor components, arterial baroreceptors were stimulated by increasing arterial pressure with a bolus injection of 1-3 μg phenylephrine (i.v., Neo-Synephrine. Sterling, Canada). Discharge of all postganglionic nerves was shown to be inhibited by baroreceptor activation.

Cervical Spinal Cord Transection.

In one group of animals (n=23), the spinal cord was exposed by a dorsal laminectomy and transected at the first cervical segment. Neural discharge, arterial pressure and heart rate were recorded from 2 min before to 5 min after spinal cord

transection and then for periods of 2 min at 15, 30 and 60 min after transection. After cord transection, phenylephrine (0.1-0.2 μg/min of 0.2 mg/ml in saline) was infused intravenously at a rate to maintain arterial pressure at control levels. Support of pressure was necessary as unsupported arterial pressure after cord transection often fell as low as 50 mmHg and the condition of the spinal cord and the animal would have deteriorated at such a low perfusion pressure.

Since preganglionic splanchnic and white rami nerve bundles may contain some postganglionic fibres (Celler & Schramm, 1981; Hayes *et al.*, 1990a), a bolus injection of a ganglionic blocking drug, hexamethonium (30 mg/kg,i.v., Sigma Chemical Company, USA), or chlorisondamine (5 mg/kg,i.v., Ciba Geigy Ltd., Canada), was given before spinal cord transection to eliminate postganglionic nerve activity when responses of these nerves were tested. In order to maintain arterial pressure at levels comparable to those before ganglionic blockade and to assure adequate perfusion of vital tissues, the infusion of phenylephrine was begun immediately after blockade in these animals and continued for the duration of the experiment. Therefore, only the responses of the preganglionic fibres to cord transection were observed.

Bilateral microinjections of glycine into the RVLM.

In another group of animals (n =11), a portion of the interparietal bone was removed to expose the cerebellum overlying the medulla. The exposed surface of the brain was kept moist with saline-soaked gauze until immediately before microinjection. Glass micropipettes were filled with a solution of the inhibitory amino acid, glycine in distilled water (1.0 M; pH=7.4; BDH Chemicals, Canada). A few

drops of India ink were added to 10 ml of the solution to mark the injection site in the brain. The tips of the pipettes were positioned in the RVLM according to the stereotaxic atlas of Paxinos and Watson (1986) and glycine was injected by application of pressurized pulses of air to the pipette. Ejection pressure and pulse duration were controlled by a picospritzer (General Valve Corporation, U.S.A.). Injection volumes were determined by the displacement of the meniscus at the air-liquid interface in the pipette observed through a microscope containing an ocular micrometer. Unilateral injections of glycine (180 nl) were made on both sides of the medulla at sites that elicited a depressor response. A recovery period of 65 min was allowed after each unilateral injection and then bilateral injections were made into these same sites. This recovery interval has been found to be sufficient for 2 consecutive glycine injections into the same site to produce identical arterial pressure and nerve responses (Hayes & Weaver, 1990).

Unilateral microinjections of glycine into the region of the A5 cell group.

A third group of animals was anaesthetized with either urethane (n =3) or anaesthetized initially with sodium pentobarbital (n =5; 40 mg/kg, i.p., M.T.C. Pharmaceutical, Canada) followed by an infusion of the steroid anaesthetic Saffan (alphaxalone 9 mg/ml and alphadolone 3 mg/ml, Glaxovet, Glaxo Canada Ltd., Canada) via a second venous cannula. The Saffan infusion (0.4-1.0 ml/hr) was begun as effects of the pentobarbital disappeared and adjustments in the rate of infusion were made as needed to maintain a constant plane of anaesthesia. In some animals, the muscarinic antagonist atropine (1 mg/kg, bolus i.v., Sigma Chemical Company, USA)

was given at 2 hr intervals to ensure that any observed changes in heart rate were due to changes in cardiac sympathetic nerve activity. A section of the parietal bone was removed to expose the cortex overlying the pons. The tips of glass micropipettes filled with a solution of glycine (1.0M; pH =7.4) were positioned within the ventrolateral pons (Paxinos & Watson, 1986) and small unilateral injections of glycine (55-70 nl) were made into sites located in the region of the A5 cell group.

Experimental protocol.

Neural discharge of renal, splenic and greater splanchnic nerves as well as arterial pressure and heart rate were recorded from 1 min before to 5 min after bilateral or unilateral injections of glycine into the RVLM or region of the A5 cell group. A recovery recording was taken when nerve discharge and arterial pressure had returned to control values. In experiments in which greater splanchnic nerve activity was recorded, hexamethonium or chlorisondamine was given to eliminate postganglionic nerve activity after a site had been found on each side of the medulla in which glycine produced a neural and blood pressure response. Arterial pressure was supported by phenylephrine infusion after ganglionic blockade. Glycine was injected into the same sites to observe neural responses of the preganglionic fibres only.

At the end of the experiments, the animals were given an overdose of urethane and background electrical noise was recorded for 2 min. Examination of the spinal cord at the location of the transection confirmed the completeness of transection. The brains were removed and stored in a solution of 10% Formalin in saline. Transverse sections of 50 μm were cut on a cryostat, stained with neutral red dye and the sites of

injection were verified using a Leitz microscope (Wild - Leitz, Canada).

Neural discharge of all postganglionic nerves and preganglionic splanchnic nerves was recorded on magnetic tape, digitized, rectified and integrated cumulatively during 10 s periods. After subtracting integrated background electrical noise, integrated nerve activity was expressed in $\mu V \cdot s$. White ramus activity was quantified in counts of spikes/s after window discrimination (Frederick Haer, U.S.A.) as outlined in previous studies (Hayes *et al*, 1990a; Hayes & Weaver, 1990).

Statistical analysis

A one-way analysis of variance (ANOVA) with repeated measures was used to determine statistical changes in neural discharge and arterial pressure after cervical spinal cord transection and bilateral injection of glycine into the RVLM or unilateral glycine injection into the A5 area (Snedecor & Cochran, 1980). Comparison between percent changes in nerve activity between groups was made using a one-way ANOVA and the Student Newman-Keuls test after square root normalization of percentage values. Differences were considered significant when $p<0.05$ and variability was expressed as pooled standard error derived from ANOVA, as a coefficient of variation or as a standard error of the mean.

3.4 Results

3.4.1 Sympathetic responses to stimulation of arterial baroreceptors

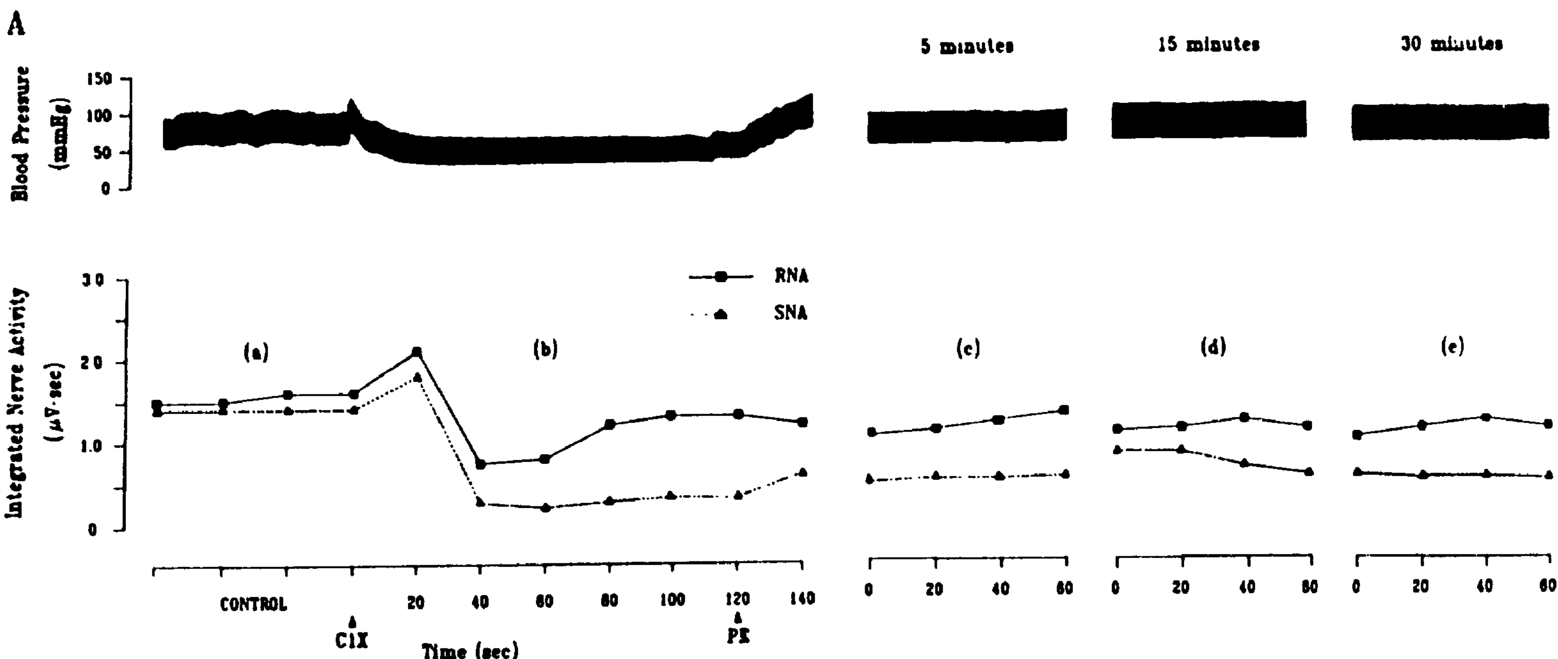
Influences of arterial baroreceptors on discharge of all postganglionic nerves (n=19) were tested in each experiment. Injections of phenylephrine (1-3 μg, i.v.) increased mean arterial pressure by 64 ±8 mmHg and inhibited ongoing discharge of renal nerves by 81 ±7% and splenic nerves by 67 ±8%. As described previously (Hayes & Weaver, 1990) renal nerves are more sensitive to activation of baroreceptors than are splenic nerves. Since activity of all nerve bundles was inhibited by stimulation of baroreceptors, these nerves were considered likely to contain vasomotor fibres.

3.4.2 Sympathetic responses to cervical spinal cord transection

Immediately following spinal cord transection, arterial pressure and activity of renal and splenic nerves increased sharply and then quickly decreased to levels significantly below control (Figs. 10 and 11). Typically, renal nerve discharge returned toward control within a few minutes of transection while splenic nerve activity remained low. By 30 min after cord transection, activity in both nerves had stabilized. Mean discharge of 6 renal nerves was decreased by 27 ±2% and mean discharge of 5 splenic nerves was decreased by 48 ±7% (Fig. 13). The decrease in renal nerve activity was significantly smaller than the decrease in splenic nerve activity.

After spinal cord transection, activity of 5 preganglionic greater splanchnic nerves was consistently decreased by 32 ±5% (Figs. 11 and 13). However, responses of preganglionic T_{13} white rami to cord transection were varied. The activity of 2 of 9

Figure 10. Response of one rat to cervical spinal cord transection (C1X). A: systemic arterial blood pressure is illustrated in the top panel. Integrated activity from renal and splenic nerves (recorded simultaneously is illustrated in the second panel. Time is indicated beneath these panels. PE indicates start of phenylephrine infusion, which was continued throughout the experiment. Letters above each panel refer to time period in which neurograms in part B were taken. B: neurogram of renal (RNA;■) and splenic (SNA;Δ) sympathetic nerves before (a) and 60 s (b), 5 min (c), 15 min (d) and 30 min (e) after cord transection. Neurograms c and d were taken at the end of each sample period. Vertical calibration is 20 μV.


A
5 minutes
15 minutes
30 minutes
Blood Pressure
(mmHg)
150
100
50
0
Integrated Nerve Activity
(μV·sec)
3.0
2.0
1.0
0
RNA
SNA
(a)
(b)
(c)
(d)
(e)
CONTROL
CIX
20
40
60
80
100
120
140
PE
Time (sec)
0
20
40
60

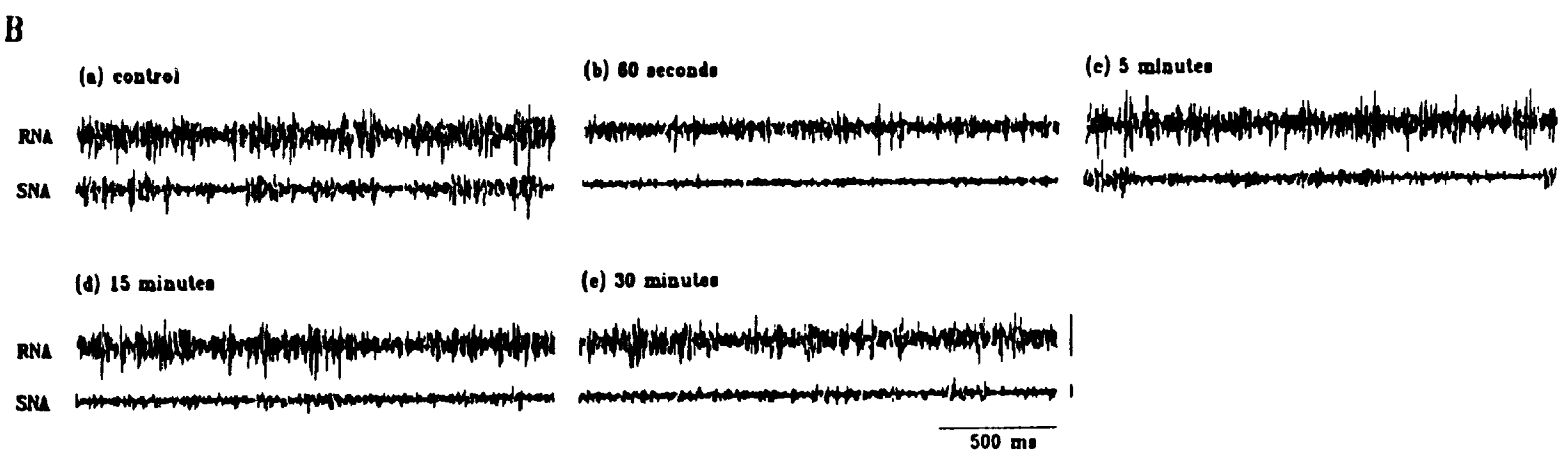
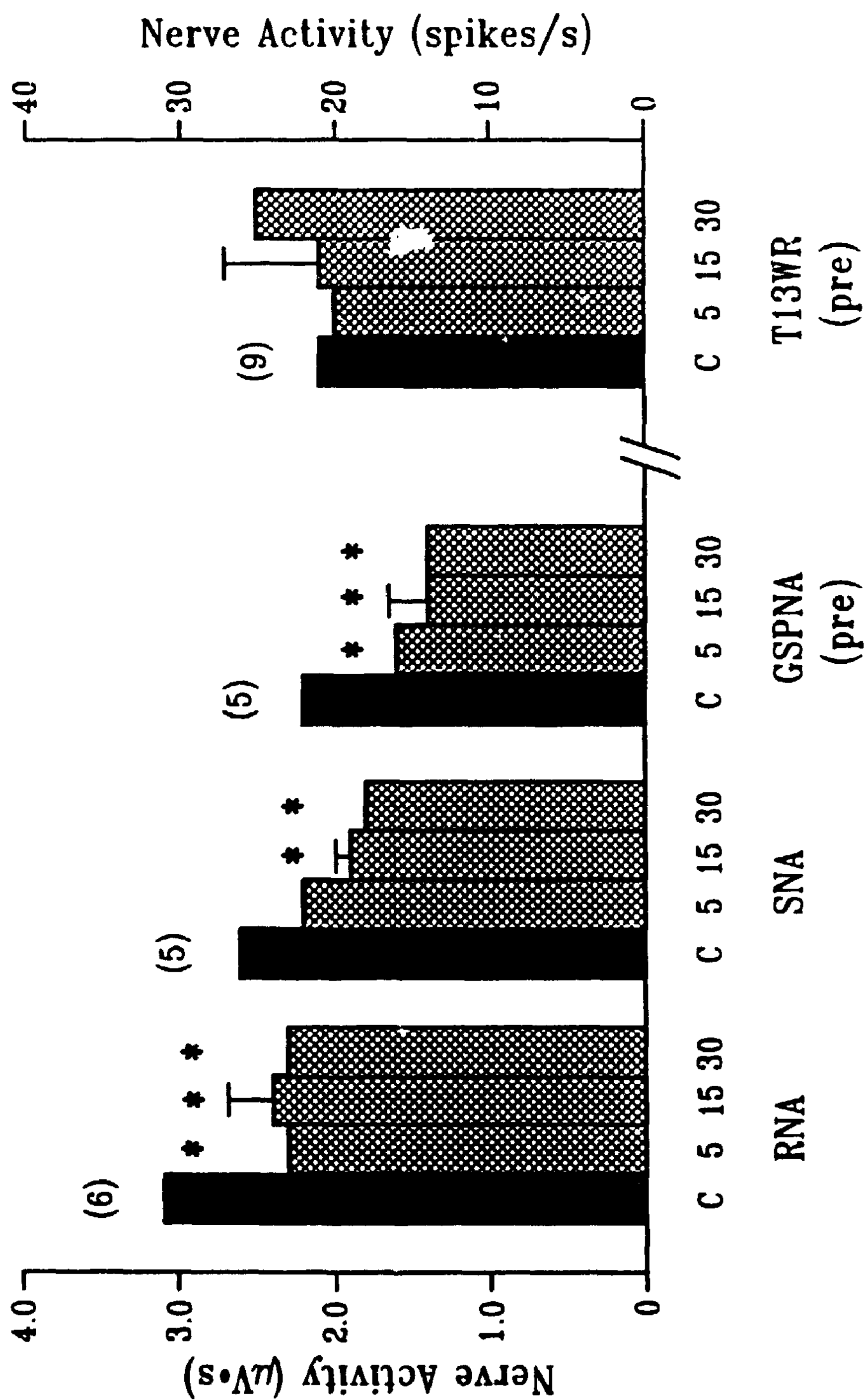

B
(a) control
(b) 60 seconds
(c) 5 minutes
RNA
SNA
(d) 15 minutes
(e) 30 minutes
RNA
SNA
500 ms

Figure 11. Mean responses of renal (RNA), splenic (SNA), preganglionic greater splanchnic (GSPNA) and preganglionic T_{13} white ramus (T_{13}WR) sympathetic nerves to cervical spinal cord transection. The filled bars are average discharge during 60 s control (C) periods. The cross-hatched bars are average discharge 5, 15 and 30 min after cord transection. Variability is indicated by the pooled standard error and asterisks indicate significant difference from control. Numbers of animals are given in parentheses. Significant decreases in nerve activity following cord transection were seen in renal, splenic and greater splanchnic nerves. Cord transection caused no significant change in T_{13} white ramus discharge.



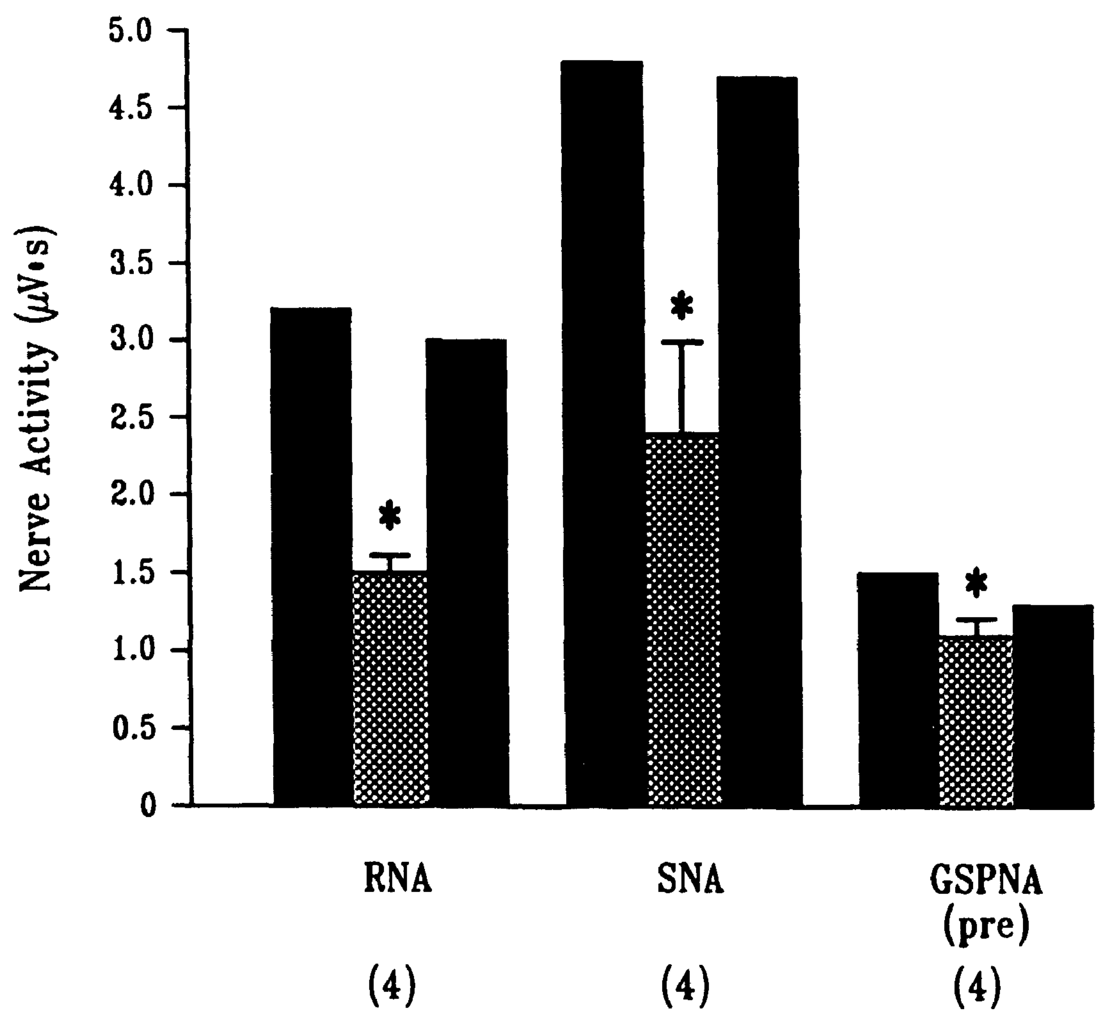
Nerve Activity (spikes/s)
40
30
20
10
0
Nerve Activity (μV•s)
4.0
3.0
2.0
1.0
0
(6)
(5)
(5)
(9)
C 5 15 30
RNA
C 5 15 30
SNA
C 5 15 30
GSPNA
(pre)
C 5 15 30
T13WR
(pre)

T_{13} white rami increased following cord transection while discharge of 3 white rami decreased and activity of 4 white rami bundles did not change. The mean change in firing of these nerve bundles (+12 ±28%) was not significant (Figs. 11 & 13). Mean control arterial pressure and heart rate for all animals (n=23) were 86 ±3 mmHg and 464 ±9 bpm before cord transection. After transection arterial pressure, supported by phenylephrine infusion was 82 ±3 mmHg and heart rate was decreased to 415 ±8 bpm.

3.4.3 Bilateral microinjections of glycine into the RVLM

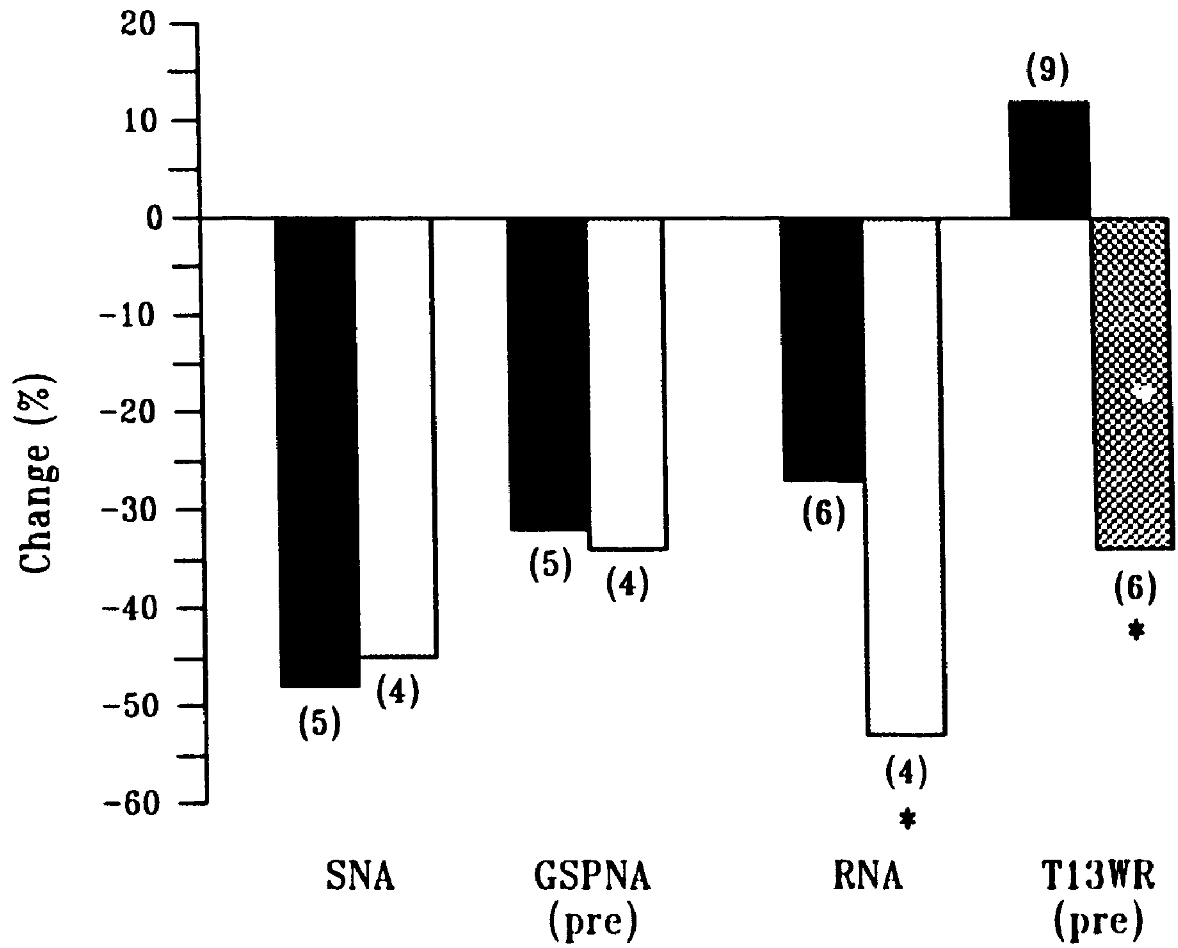
Bilateral injection of glycine into the RVLM produced a large decrease in arterial pressure as well as changes in renal and splenic nerve activity that began within seconds and maximum decreases occurred within 1-5 min of injection. Arterial pressure and nerve activity returned to control values within 30 min to 1 hr. Since arterial pressure began to return to control values within minutes of the maximum decrease, support of pressure during this period was not necessary. Mean control arterial pressure and heart rate for these animals (n=7) were reduced significantly by 42 ±4% (from 86 to 49 mmHg; pooled S.E. = 3.9) and by 10 ±2% (from 481 to 431 bpm; pooled S.E. = 4.6). Bilateral glycine injection decreased renal nerve activity by 53 ±5%, and decreased splenic nerve activity by 45 ±6% (Figs. 12 and 13). Bilateral injections of glycine into the RVLM reduced preganglionic greater splanchnic nerve activity by 34 ±5% (Figs. 12 and 13) and had no effect on arterial pressure and heart rate because of the ganglionic blockade. Responses of T_{13} white rami to bilateral RVLM injections were not tested as these recordings could not be maintained for the three hours needed to test responses of preganglionic nerves.

Figure 12. Mean responses of renal, splenic and greater splanchnic nerves to bilateral injections of glycine into the RVLM. Filled bars are average discharge during 60 s control and recovery periods. The cross-hatched bars are average discharge during the 10s period of maximum response to glycine injection. Remainder of format is as in **Figure 11**. Bilateral RVLM blockade caused significant decreases in discharge of all three nerves.



Nerve Activity (μV•s)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
*
*
*
RNA
SNA
GSPNA
(pre)
(4)
(4)
(4)

Figure 13. Comparison of neural responses to cervical spinal cord transection with responses to blockade of the RVLM. All values are expressed as a percentage change from control. Changes from control 30 min after cord transection are shown as filled bars. Maximal responses to bilateral blockade are illustrated as stippled bars. Maximal response of T_{13} white rami to unilateral blockade (cross-hatched bar) has been taken from a previous study (Hayes & Weaver, 1990). Numbers in parentheses are numbers of injections (RVLM blockade) or animals (cord transection). Asterisks indicate significant difference between responses to spinal cord transection and RVLM blockade. Coefficients of variation are 0.20 (SNA), 0.18 (GSPNA), 0.10 (RNA) and 1.85 (T_{13}WR). Responses of splenic and splanchnic nerves to cord transection were not different from those to bilateral blockade. In contrast, renal and T_{13} white ramus responses to spinal cord transection were smaller than those to bilateral or unilateral medullary blockade, respectively.


20
10
0
-10
-20
-30
-40
-50
-60
Change (%)
(9)
(5)
(4)
(5)
(4)
(6)
(4)
*
(6)
*
SNA
GSPNA
(pre)
RNA
T13WR
(pre)

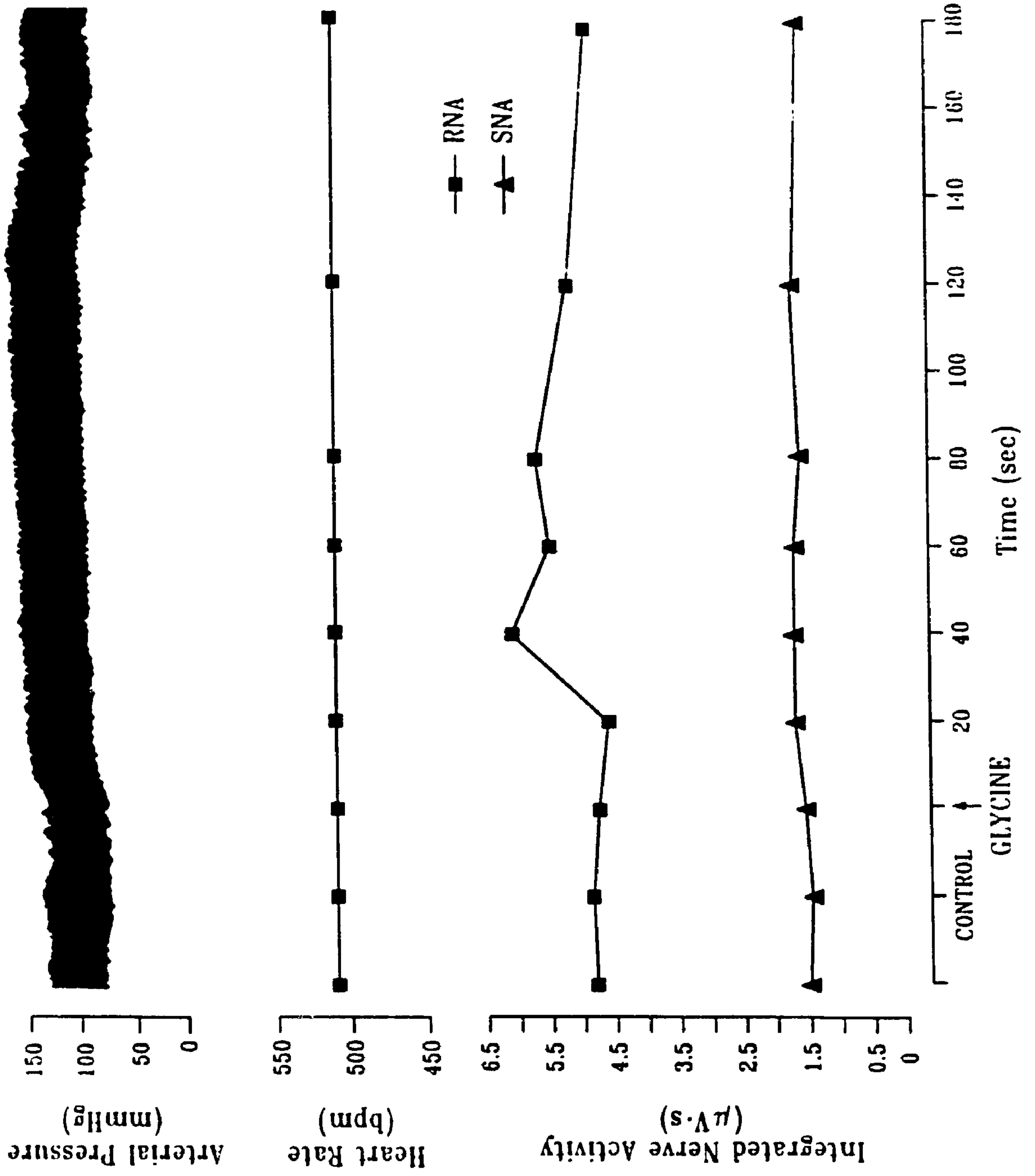
3.4.4 Comparison of cervical spinal cord transection and RVLM blockade

A comparison of responses to cervical spinal cord transection and RVLM blockade is shown in Fig. 13. For this comparison of T_{13} white rami responses, data for unilateral injections of glycine into the RVLM have been taken from a previous study (Hayes & Weaver, 1990). Transecting the spinal cord caused reductions in discharge of splenic and splanchnic nerves which were not different from those caused by bilateral glycine injections. In contrast, discharge of renal nerves was decreased to a greater extent by bilateral injections of glycine into the RVLM than by cervical spinal cord transection. Furthermore, even unilateral blockade of the RVLM decreased the discharge of T_{13} white rami more than spinal cord transection.

3.4.5 Unilateral microinjections of glycine into the region of the A5 cell group.

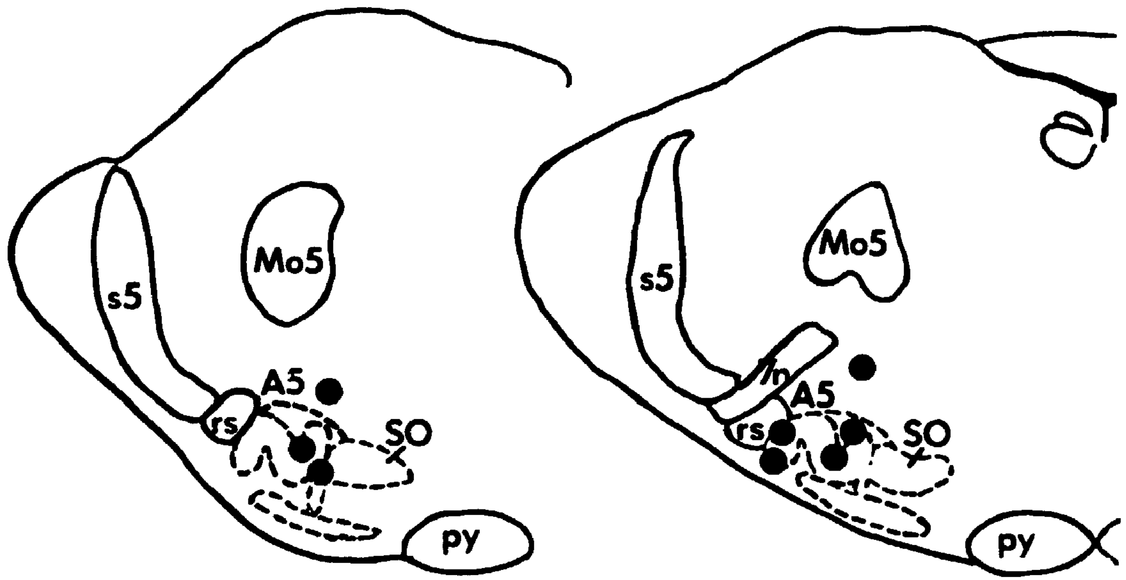
An example of the sympathetic, arterial pressure and heart rate responses produced by a unilateral injection of glycine into the A5 region is shown in Fig. 14. Unilateral glycine injections (n =8) into sites in the vicinity of the A5 cell group caused arterial pressure and sympathetic responses that began within 5-10s, reached a maximum response in 30-150s and returned to control values within 15 min. Mean arterial pressure was increased by 15 ±3 mmHg (from 85 to 100 mmHg; pooled S.E.=2.1) but there were no significant effects on heart rate. Glycine injection caused increases in renal nerve activity of 30 ±5% (from 3.1 to 3.8 μV·s; pooled S.E=1.6). In some (n =5) but not all of these sites, glycine injection also increased activity of splenic nerves by 22 ±4% (from 0.9 to 1.1 μV·s; pooled S.E.=0.37). The locations of these injection sites are shown in Fig. 15.

Figure 14. Response of one rat to a unilateral microinjection (61 nl) of glycine (1.0M) into a site near the A5 cell group. Top panel: record of systemic arterial blood pressure; Middle panel: heart rate; Bottom panel: integrated activity from renal (■) and splenic (Δ) nerves (recorded simultaneously). Time is indicated beneath these panels. Injection of glycine (arrow) produced an increase in arterial pressure and renal nerve activity but had little effect on heart rate or splenic nerve activity.



Arterial Pressure
(mmHg)
150
100
50
0
Heart Rate
(bpm)
550
500
450
Integrated Nerve Activity
(μV·s)
6.5
5.5
4.5
3.5
2.5
1.5
0.5
0
RNA
SNA
CONTROL
GLYCINE
20
40
60
80
100
120
140
160
180
Time (sec)

Figure 15. Drawings of transverse sections of rat pons 0.30 mm and 0.80 mm caudal to the interaural line showing sites of glycine injection (filled circles). s5 (spinal trigeminal tract); rs (rubrospinal tract); Mo5 (motor trigeminal nucleus); A5 (A5 cell group); SO (superior olivary nucleus); py (pyramidal tract); 7n (facial nerve).



−0.30 mm

−0.80 mm

1 mm

3.5 Discussion

The observation that bilateral RVLM blockade caused decreases in firing of renal nerves which were greater than those caused by cord transection suggests that these nerves are under descending tonic inhibitory restraint balanced by excitatory drive from the RVLM. This pattern of response was also observed in the T_{13} white rami since even unilateral RVLM blockade produced larger decreases in nerve activity than cord transection. Blockade of the RVLM eliminated excitatory input to sympathetic preganglionic neurons leaving a descending inhibitory influence on renal nerves and T_{13} white rami unopposed. This inhibitory pathway was then interrupted by spinal cord transection resulting in increased sympathetic discharge of spinal origin. Our results also suggest that tonic inhibitory influences selectively affect lower thoracic white rami and renal nerves rather than affecting sympathetic innervation of the viscera in general. In fact, anatomical and electrophysiological studies have shown that renal postganglionic neurons receive preganglionic input from T_{13} white rami and that splenic neurons are innervated primarily by the greater splanchnic nerve (Ferguson *et al.*, 1986; Sripairojthikoon & Wyss, 1987; Chevendra & Weaver, 1991; Taylor & Weaver, 1992a). This indicates that this tonic inhibitory system affects the sympathetic pathway to the kidney and not the pathway to the spleen.

The greater renal nerve responses to RVLM blockade than to spinal cord transection may also be consistent with an alternative explanation. Renal nerves may be more sensitive to RVLM blockade than splenic nerves, not due to intact descending inhibition of renal nerve activity but because of a pattern of organization of neurons in

the RVLM leading to differential control of these two nerves. Renal nerve discharge is also more sensitive to baroreceptor activation, a reflex which is mediated, at least in part by neurons in the RVLM (Granata *et al.*, 1985a). In addition, preganglionic neurons controlling renal nerves may be more capable of maintaining sympathetic firing after spinal cord transection than are those innervating splenic nerves. Possibly, neither responses to RVLM blockade nor those to cord transection depend upon the presence of an inhibitory system.

Several regions of the brainstem have been considered as sources of tonic sympathoinhibition. Neurons in the medullary raphe nuclei and caudal ventrolateral medulla (CVLM) have both been shown to inhibit sympathetic discharge tonically (McCall & Harris, 1987; Pilowsky *et al.*, 1985; Willette *et al.*, 1984b). Although bulbospinal projections from both these areas have been reported (Loewy, 1981; Loewy & McKellar, 1981; Fleetwood-Walker *et al.*, 1983), recent evidence suggests that these regions influence sympathetic activity by tonically inhibiting sympathoexcitatory neurons in the RVLM (Willette *et al.*, 1984b; Granata *et al.*, 1986; McCall, 1988a; Agarwal *et al.*, 1989). Since our results suggest an inhibitory system not mediated through these excitatory RVLM neurons, the CVLM or raphe nuclei are not likely sources of the inhibition observed in this study. Even baroreceptor-mediated inhibition was probably interrupted by the bilateral RVLM injections as this reflex is largely relayed through RVLM neurons (Granata *et al.*, 1985a).

In the present study, injections of glycine into sites in the region of the A5 cell group caused increases in arterial pressure and sympathetic nerve activity suggesting

that A5 neurons tonically inhibit vasomotor discharge. Although recent evidence indicates that A5 neurons may be sympathoexcitatory (Huangfu *et al.*, 1991; Huangfu *et al.*, 1992), our results are consistent with those of other studies that support a sympathoinhibitory role for A5 neurons (Stanek *et al.*, 1984; Loewy *et al.*, 1986). Since A5 noradrenergic neurons are dispersed amoung reticular neurons within the ventrolateral pons, cardiovascular and sympathetic responses may have been caused by an action of glycine on neurons other than A5 cells. However, this possibility is unlikely because glycine injection into sites throughout the pontine reticular formation produces consistent decreases in arterial pressure and renal and splenic sympathetic nerve activity (Hayes & Weaver, 1992). Since glycine injections into this region increased discharge of renal and splenic nerves but had little effect on cardiac sympathetic nerve activity, this inhibition could be specific for some sympathetic nerves. However, since glycine injections into this area produced similar responses in both renal and splenic nerves, the A5 cell group may not be the source of selective inhibition of renal nerves that is suggested by the results of this study.

Several studies have revealed a baroreceptor-independent inhibitory system that descends in the dorsolateral funiculus of the spinal cord (Dembowsky *et al.*, 1980; Müller *et al.*, 1988). Following spinal cord transection, electrical stimulation of the dorsolateral funiculus causes a reduction in arterial pressure and in sympathetic nerve activity (Coote & Macleod, 1974; Schramm & Chornoboy, 1982; Schramm & Livingstone, 1987). Although stimulation of this pathway reduces renal nerve activity by 50%, stimulation has little effect on lumbar sympathetic discharge (Taylor &

Schramm, 1988), suggesting that the inhibition is selective for certain sympathetic nerves. Schramm and Livingstone's (1987) hypothesis that this inhibitory system has the strongest effect on nerves capable of generating activity after spinal cord transection is supported by the fact that, in chloralose-anaesthetized rats, renal and gastrosplenic nerve activity increases after cord transection, whereas lumbar chain discharge decreases (Taylor & Schramm, 1987; 1988). Our results do not support this idea because even though splanchnic nerve activity was maintained as well as that of renal nerves in spinal rats, we found no evidence that splanchnic nerve activity was tonically inhibited.

Another difference between our results and those of Taylor and Schramm (1987; 1988) is that spinal cord transection consistently decreased activity of both renal and splenic nerves in our urethane-anaesthetized rats. To investigate the possibility that the differences in the renal nerve responses between these studies were due to the different anaesthetics used, we did four additional experiments in rats anaesthetized with alpha-chloralose. In 2 of these rats, renal nerve discharge did decrease. However, we were able to produce increases in renal nerve activity with cord transection in 2 of these rats suggesting that responses to cord transection can be affected by the anaesthetic used.

In all of the nerves studied, a substantial amount of activity (50% or more) remained after bilateral blockade of neurons in the RVLM or even after cervical spinal cord transection. The origin of this sympathetic firing is unknown. Sympathetic reflexes can be elicited by activation of a variety of visceral afferent nerves in spinal

animals (Meckler & Weaver, 1988; Stein & Weaver, 1988). In preliminary experiments in our laboratory, blockade of afferent nerve activity by cooling the intestine produced decreases in mesenteric nerve activity before (35%) and after (16%) spinal cord transection in rats suggesting that tonic excitatory afferent inputs may provide some ongoing activity of these nerves. However, since dorsal root rhizotomy failed to reduce mesenteric nerve activity in rats with intact or transected spinal cords, peripheral afferent inputs to the spinal cord probably do not contribute to tonic excitation of mesenteric neurons (Taylor & Weaver, 1992b).

Sympathetic discharge is probably initiated within spinal cord propriospinal circuits after cord transection but the importance of these circuits for ongoing sympathetic activity when the neuraxis is intact is unknown. Spinal networks may normally play a minor role in maintaining ongoing sympathetic discharge and become more important as a source of tonic drive after cord transection. However, activity in splanchnic and splenic nerves following bilateral RVLM blockade is similar to activity remaining in these nerves after cervical cord transection. This may indicate that, even in the intact animal spinal generators make a substantial contribution to ongoing sympathetic discharge to the viscera.

In summary, these experiments have identified another characteristic of sympathetic control which could provide selective, non-uniform influences on individual visceral organs such as the kidney and spleen. Thirteenth thoracic white rami and renal nerves are not only more dependent upon excitatory drive from the RVLM than are splenic, mesenteric and preganglionic greater splanchnic nerves (Hayes & Weaver, 1990), but

appear to be the target of tonic inhibition. This inhibition could not be produced by actions on RVLM neurons and may be caused by projections of tonically active neurons to the spinal cord which selectively affect the sympathetic pathway to the kidney. Although neurons located in the region of the A5 cell group appear to tonically inhibit activity of some sympathetic nerves, sympathoinhibition from this region is not specific for renal nerves.

Chapter 4: Tonic sympathetic excitation and vasomotor control from pontine reticular neurons in rats.

American Journal of Physiology 263: H1567-H1575, 1992.

4.1 Introduction

Efforts in the last 10 years to locate specific brainstem nuclei involved in tonic vasomotor control have focused on the rostral ventrolateral medulla (RVLM) and evidence has accumulated that supports the RVLM as the most critical site for cardiovascular control and for generation of sympathetic activity (review: Calaresu & Yardley, 1988; Barman, 1990; Guyenet, 1990; McCall, 1990; Chalmers & Pilowsky, 1991). However, in light of recent studies, the idea of RVLM neurons as the only crucial generator of sympathetic vasomotor tone requires serious reevaluation. For example, extensive bilateral electrolytic lesions of the RVLM in anaesthetized rats produced only transient (30 minutes) decreases in arterial pressure (Cochrane *et al.*, 1988). Furthermore, bilateral electrolytic or chemical RVLM lesions in conscious rats did not affect basal arterial pressure or heart rate and did not eliminate the baroreflex cardiac response to a rise in arterial pressure (Cochrane *et al.*, 1989; Vasquez *et al.*, 1992). These studies indicate that although the RVLM remains an important region in cardiovascular control, areas outside of the RVLM are capable of maintaining arterial pressure and mediating cardiovascular reflexes.

Other regions including the forebrain (Huang, Gebber, Barman & Varner, 1987), the hypothalamus (Huang, Varner, Barman & Gebber, 1988) and even the spinal cord

(Dembowsky *et al.*, 1985; Meckler & Weaver, 1985; 1988; Taylor & Schramm, 1987; Stein & Weaver, 1988) have been suggested as potential contributors to vasomotor tone. The dorsal medullary reticular formation was once considered a generator of basal sympathetic discharge since lesions within this region lower blood pressure in anaesthetized rats (Chai & Wang, 1968; Kumada, Dampney & Reis, 1979). Since subsequent experiments showed that these lesions interrupted a pathway mediating responses from the RVLM, this region was prematurely dismissed as a primary source of sympathetic activity (Dampney & Moon, 1980). Several recent studies suggest that a network of neurons in the medullary reticular formation integrates cardiovascular reflexes and maintains tonic sympathetic nerve activity. Injections of excitatory amino acids into the dorsal and dorsomedial medulla cause pressor responses and vasoconstriction in the renal vascular bed suggesting that neurons involved in vasomotor functions exist in this area (Chai, Lin, Lin, Pan, Lee & Kuo, 1988; Yardley, Andrade & Weaver, 1989b). Furthermore, injections of the inhibitory amino acid glycine can produce decreases in systemic arterial pressure and renal sympathetic nerve activity (Korkola & Weaver, 1992). Barman and Gebber (1987; Gebber & Barman, 1988) have located neurons in the lateral tegmental field (LTF) of cats with spontaneous activity synchronized to the cardiac cycle and to the 2-6 Hz rhythm of sympathetic nerve discharge. The firing rate of some of these neurons decreases during baroreceptor reflex activation suggesting that these neurons have sympathoexcitatory functions. Although LTF neurons with sympathetic related activity do not send axons to the thoracic spinal cord (Gebber & Barman, 1985) these neurons

do send excitatory projections to the RVLM (Barman & Gebber, 1987) and studies from their laboratory suggest that LTF neurons are an important source of basal activity for RVLM-spinal sympathoexcitatory neurons. This conclusion is based on the finding that sympathoexcitatory neurons in the LTF fire significantly earlier than RVLM neurons during the 2-6 Hz burst of sympathetic nerve discharge (Barman & Gebber, 1987; Gebber & Barman, 1988). In addition, the conduction time in the pathway from the LTF to the RVLM corresponds with the difference in the spontaneous firing times of the two groups of neurons during the 2-6 Hz burst of sympathetic discharge (Barman & Gebber, 1987; Gebber & Barman, 1988).

To consider the medullary reticular formation a vasomotor centre it must be a site of convergence of major inputs from other cardiovascular regulatory regions (Calaresu & Yardley, 1988). Single neurons in the reticular formation respond to baroreceptor input, to both noxious and innocuous stimuli applied to the heart and are excited by electrical stimulation of cardiac sympathetic afferents (Blair, 1987). In addition to cardiovascular information these neurons also respond to a wide variety of inputs including somatic stimuli, auditory and visual stimuli, activation of respiratory afferents and input from vestibular, cortical, tectal and cerebellar pathways. Most of these reticular neurons receive convergent input from multiple sources (review: Foreman & Blair, 1988). Therefore the function of this area may be to integrate and process visceral, sensory and emotional or behavioral information and then to provide the appropriate cardiovascular responses.

Specific nuclei in the pons have been implicated in various autonomic functions

including micturition (Holstege, Griffiths, Wall & Dalm, 1986), respiration (Feldman & Ellenberger, 1988) and cardiovascular regulation (Mraovitch, Kumada & Reis, 1982; Gurtu, Pant, Sinha & Bhargava, 1984; Ward, 1988; Lai & Siegel, 1990). The A5 cell group, a collection of noradrenergic cells in the ventrolateral portion of the pons has been shown to be involved in control of the sympathetic nervous system. Injections of excitatory amino acids into this region have been reported to cause either sympathoexcitation (Huangfu *et al.*, 1992) or sympathoinhibition (Stanek *et al.*, 1984) accompanied by decreases in arterial pressure and heart rate. In addition, approximately 93% of these neurons send axons to the intermediolateral cell column of the spinal cord (Byrum *et al.*, 1984). However, the remainder of the pontine reticular formation is largely unexplored. Neurons in the medial PRF are thought to mediate events of desynchronized sleep including rapid eye movements and muscle twitches (McCarley, 1980) and stimulation of some ventrolateral PRF sites produces antinociception (Miller & Proudfit, 1990). Anatomical studies have demonstrated projections from lateral PRF neurons to the RVLM (Ruggiero *et al.*, 1989). PRF neurons do not project to the sacral spinal cord (Holstege *et al.*, 1986) indicating that they are indeed a separate population from "micturition neurons" in the dorsal pons (Loewy, Saper & Baker, 1979b). In a 1939 study, electrical stimulation of pontine reticular neurons caused pressor responses (Wang & Ranson, 1939) but, to our knowledge, there have been no recent studies on the potential role PRF neurons may have in cardiovascular and sympathetic control.

4.2 Rationale for the present study

We propose that the neural organization providing basal levels of sympathetic discharge is not limited to the RVLM but entails a complex and more extensive network of neurons. The present investigation was done to explore our empirical finding that neurons in the pontine reticular formation make a substantial contribution to resting control of arterial pressure, heart rate and activity of sympathetic nerves. Tonic cardiovascular control by this region was compared to control by a more well-defined cardiovascular area, the RVLM.

4.3 Methods

Surgical procedures.

Male Wistar rats (250-350 g; Charles River, Canada) were anaesthetized initially with sodium pentobarbital (40 mg/kg, i.p., M.T.C. Pharmaceutical, Canada) and a tracheal tube was inserted. Both jugular veins and the right femoral artery were cannulated for delivery of solutions and drugs and for monitoring arterial blood pressure, respectively. Arterial blood samples (~100 μl) were withdrawn periodically for analysis of pH, pO_2 and pCO_2 (pH-blood gas analyser, model 170, Corning Medical, USA) and deviations from normal were corrected by i.v. administration of sodium bicarbonate or by adjusting respiratory rate and/or tidal volume. Body temperature was maintained at 37°C with a heating pad. An infusion of the steroid anaesthetic Saffan (alphaxalone 9mg/ml and alphadolone 3mg/ml, Glaxovet, Glaxo Canada Ltd, Canada) via one venous cannula (0.4-1.0 ml/hr) was begun as effects of the pentobarbital disappeared and adjustments in the rate of infusion were made as needed to maintain a constant plane of anaesthesia. The animal's plane of anaesthesia was assessed by examining palpebral and withdrawal reflexes. A solution of physiological saline was infused slowly (≤0.6 ml/hr) through the other venous cannula to compensate for fluid loss. The animals were artificially respired with oxygen-enriched air and paralysed with gallamine triethiodide (20 mg/kg initially, followed by doses of 10 mg/kg as needed, Rhone-Poulenc, Canada). Before each supplemental dose of gallamine was given, the animal's plane of anaesthesia was assessed. The rats were placed in a stereotaxic frame (David Kopf Instruments, USA) and sections of the

parietal and interparietal bones were removed to expose the cortex and cerebellum overlying the pons and medulla. The surface of the brain was kept moist with saline-soaked gauze.

Neural recordings.

The spleen and left kidney were exposed by a left flank incision and small postganglionic nerve bundles were dissected from the renal and splenic arteries and severed. The central ends were placed on small stainless-steel bipolar electrodes and covered with dental impression medium (Perfourm, Cutter Dental, USA) to isolate the nerve and electrode from the surrounding tissue and to prevent dehydration. Multifibre discharge from both nerves was recorded simultaneously (n=33) and was amplified at a bandwidth of 30 Hz-3 kHz. In 9 animals, only discharge of renal nerves was recorded and in 1 animal only discharge of splenic nerves was recorded. The amplified signals were monitored on an oscilloscope, recorded on magnetic tape and displayed with arterial pressure and heart rate on a Grass Instruments polygraph (Grass Instrument Company, USA). A pneumothorax was made to eliminate artifacts in the neural recordings caused by respiratory movements. Before and throughout the experiment, the muscarinic antagonist atropine (1 mg/kg, bolus,i.v., Sigma Chemical Company, USA) was given at 2 hr intervals to ensure that changes in heart rate were due to changes in cardiac sympathetic activity. To ensure that recordings were from sympathetic nerves containing vasomotor components, arterial baroreceptors were stimulated by increasing arterial pressure with a bolus i.v. injection of 1-3 μg phenylephrine (Neo-Synephrine, Sterling, Canada) to elicit decreases in heart rate and

sympathetic nerve activity.

Microinjection procedures.

Glass micropipettes were pulled to a tip size of about 40 μm and filled with a solution of the inhibitory amino acid glycine (0.001M, 0.01M, 0.1M or 1.0M; pH=7.4, BDH Chemicals, Canada). The 0.001M and 0.01M solutions contained glycine dissolved in normal physiological saline and the 0.1M and 1.0M solutions contained glycine dissolved in distilled water. India ink (150 μl) was added to 10 ml of the solution to mark the injection site in the brain. The tips of the pipettes were positioned within the pontine reticular formation (PRF) according to the stereotaxic atlas of Paxinos & Watson (1986). Glycine (55-85 nl) was microinjected unilaterally into the PRF of 22 rats using a picospritzer (General Valve Corporation, USA). Injection volumes were determined by the displacement of the meniscus at the air-liquid interface in the pipette observed through a microscope containing an ocular micrometer. Arterial pressure, heart rate and discharge of renal and splenic nerves were recorded from 1 min before to 5 min after the beginning of microinjection into the PRF. To determine the most effective concentration of glycine for use in this study, a dose-response relationship for glycine was established in 9 rats by injecting 0.001M, 0.01M, 0.1M and 1.0M glycine into the pons. Up to 3 injections were made into the same site, administering different concentrations randomly. In exception to this random design, the 1.0M concentration was not usually injected before testing the 2 lowest concentrations. These glycine injections were separated by periods of 65 min because a previous study showed that 2 consecutive glycine injections into the same

site could elicit equal responses if this minimum time period was allowed between injections (Hayes & Weaver, 1990). No other procedures were done in these 9 rats. Since the rostral ventrolateral medulla (RVLM) is a well-established cardiovascular area, in 6 of the remaining 13 animals glycine (1.0M, 55-75 nl) was injected into sites within the RVLM and the cardiovascular and neural responses to these injections were compared to those following glycine injection into the PRF. In 3 rats, glycine (1.0M) was microinjected bilaterally (70nl each side) into the PRF. Because a microscope was positioned on each micropipette, the 2 injections could be delivered with 2 channels of the picospritzer within a 5s interval.

To determine the involvement of the baroreceptor reflex in responses evoked from the PRF, glycine (1.0M, 35-75nl) was microinjected into the PRF of 4 rats after sino-aortic denervation. Sino-aortic denervation was accomplished by severing the ninth and tenth cranial nerves bilaterally as they entered the posterior lacerated foramen of the cranium. Complete denervation was assumed if the elevation in arterial pressure caused by a bolus i.v. injection of phenylephrine (1-3μg) was not accompanied by reflex decreases in heart rate and sympathetic nerve discharge.

Control injections of sodium chloride (1.0M; pH=7.4; 65-75 nl; 4 rats) or sucrose (2.0M; pH=7.4; 45-75 nl; 3 rats) were made into sites in the PRF before the same volume of glycine (1.0M) was injected into the same site. In 4 rats, the cardiovascular and sympathetic effects of excitation of PRF neurons were tested by microinjection (65-95 nl) of the glutamate agonist, D,L-homocysteic acid (DLH; 0.17M; pH=7.4). Finally, in 3 rats, the long-lasting $GABA_A$ agonist muscimol (0.9mM, pH=7.4) was

injected unilaterally (30-45 nl) into the PRF and the cardiovascular and sympathetic responses were compared to those following unilateral glycine (1.0M) injection.

At the end of the experiments, the animals were given an overdose of anaesthetic and background electrical noise was recorded for 2 min. The brainstems were removed and stored in a solution of 10% formalin in saline. Transverse sections of 50 μm were cut on a cryostat, stained with Neutral Red dye and the sites of injection were identified by India ink deposits using a Leitz microscope (Wild-Leitz, Canada) and mapped on drawings of transverse sections of the rat medulla (Paxinos & Watson, 1986).

Neural discharge recorded on magnetic tape (Racal, model 7DS, UK) was digitized, rectified and integrated cumulatively during 10 s periods. After subtracting integrated background electrical noise, integrated nerve activity was expressed as μV·s. Values of mean arterial pressure are presented throughout this study.

Statistical analyses.

Individual sympathetic or cardiovascular responses to each injection were determined using a 95% confidence interval. A one-way analysis of variance (ANOVA) with repeated measures was used to determine statistical changes in mean neural discharge, heart rate and arterial pressure after injection of solutions into the PRF or RVLM. Comparison between percentage changes in renal and splenic nerve activity was made using an independent t-test after square root normalization of percentage values. A completely random ANOVA was used to compare arterial pressure, heart rate and sympathetic nerve responses to injection of 0.001M, 0.01M,

0.1M and 1.0M glycine into the PRF. Differences between mean values in ANOVA analyses were determined using Tukey's test. Differences were considered significant when $p<0.05$. Variability was expressed by a pooled standard error calculated from the ANOVA error mean square term or as a standard error of the mean (Snedecor & Cochran, 1980).

4.4 Results

4.4.1 Sympathetic responses to stimulation of arterial baroreceptors.

All renal and splenic nerves were tested for baroreceptor sensitivity by evaluating responses to increases in arterial pressure elicited by i.v. injections of phenylephrine. An increase in mean arterial pressure from 95 ±3 to 165 ±4 mmHg significantly decreased heart rate by 23 ±3 bpm (from 375 to 352 bpm; pooled S.E.=2.2) and inhibited ongoing discharge of renal nerves by 88 ±3% (from 1.8 to 0.3 $\mu V{\cdot}s$; pooled S.E.=1.5) and splenic nerves by 83 ±4% (from 2.7 to 0.5 $\mu V{\cdot}s$; pooled S.E.=2.5). The decreases in renal and splenic nerve activity were not different from each other.

4.4.2 Microinjections of glycine into the pontine reticular formation (PRF).

An example of the sympathetic, arterial pressure and heart rate responses produced by a unilateral injection of glycine into the PRF is shown in Fig. 16. Unilateral glycine injections (n=36) into the PRF produced brisk decreases in arterial pressure, heart rate and sympathetic nerve activity that began within 5-10 seconds, reached a maximum decrease in 33 ±6s and returned to control values within 116 ±17s in most sites (n=34). Two injections into the PRF caused longer lasting (1200s) decreases in sympathetic nerve discharge. In all other respects, responses from these two sites were within the range of values obtained from the other 34 sites. Mean arterial pressure was decreased by 29 ±2 mmHg (from 110 to 81 mmHg; pooled S.E.=1.3) and heart rate was decreased by 20 ±2 bpm (from 379 to 359 bpm; pooled S.E.=1.4) following glycine injection (Fig. 17). Glycine injection caused decreases in renal nerve activity of 47 ±4% (from 2.0 to 1.1 $\mu V{\cdot}s$; pooled S.E.=0.61) that were not

Figure 16. Responses to a unilateral microinjection (65 nl) of glycine (1.0M) into the pontine reticular formation (PRF) of one rat. Top panel: record of systemic arterial blood pressure; Middle panel: heart rate; Bottom panel: integrated activity from renal (■) and splenic (Δ) nerves (recorded simultaneously). Time is indicated beneath these panels. Injection of glycine (arrow) produced large, brisk decreases in arterial pressure and equal renal and splenic nerve discharge. In this particular animal, the heart rate response was smaller than usual. The changes in arterial pressure, heart rate and sympathetic activity occurred with a short latency (20s) and recovered to control levels 60s after glycine injection.

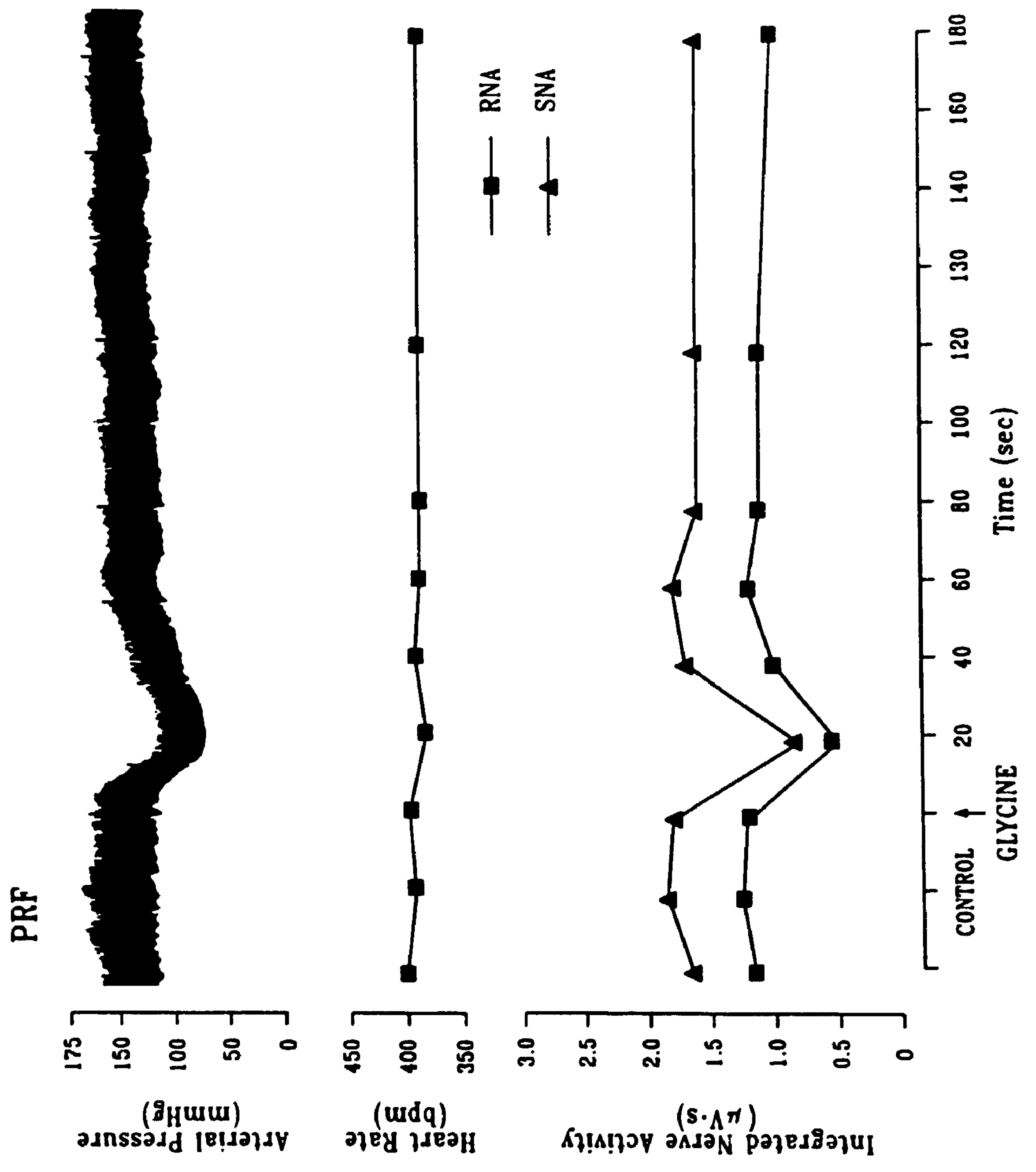
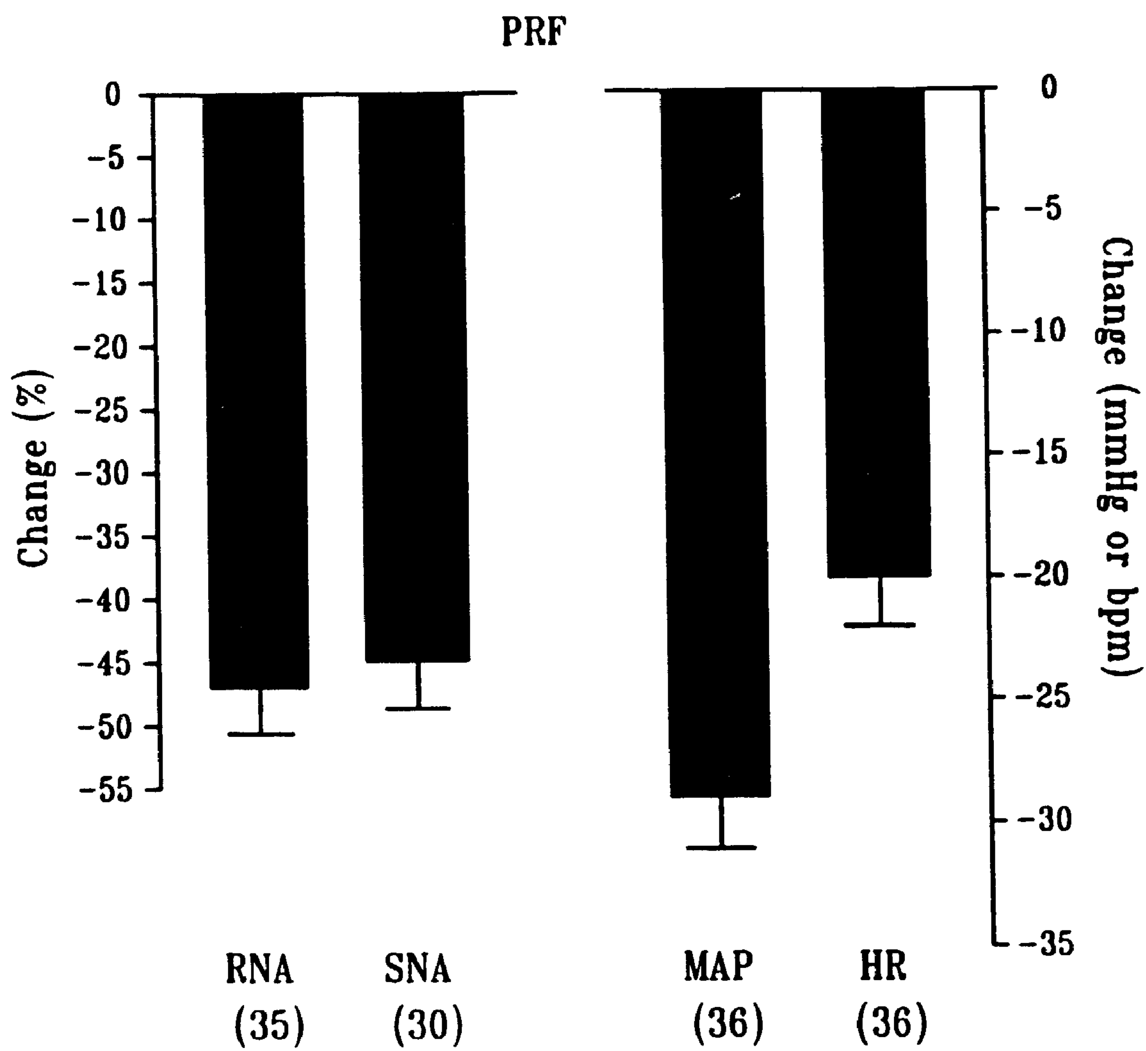

PRF
Arterial Pressure
(mmHg)
175
150
100
50
0
Heart Rate
(bpm)
450
400
350
Integrated Nerve Activity
(μV·s)
3.0
2.5
2.0
1.5
1.0
0.5
0
RNA
SNA
CONTROL
GLYCINE
20
40
60
80
100
120
130
140
160
180
Time (sec)

Figure 17. Mean changes in mean arterial pressure (MAP) and heart rate (HR; right panels) and renal (RNA) and splenic (SNA) nerve activity (left panels) caused by unilateral injections of glycine into the pontine reticular formation (PRF). Variability is indicated by the S.E.M.. Number of injections is indicated in parentheses. Glycine injections caused significant decreases in arterial pressure, heart rate and sympathetic nerve activity. The decreases in renal and splenic nerve discharge were not different.


PRF
0
-5
-10
-15
-20
-25
-30
-35
-40
-45
-50
-55
Change (%)
0
-5
-10
-15
-20
-25
-30
-35
Change (mmHg or bpm)
RNA
(35)
SNA
(30)
MAP
(36)
HR
(36)

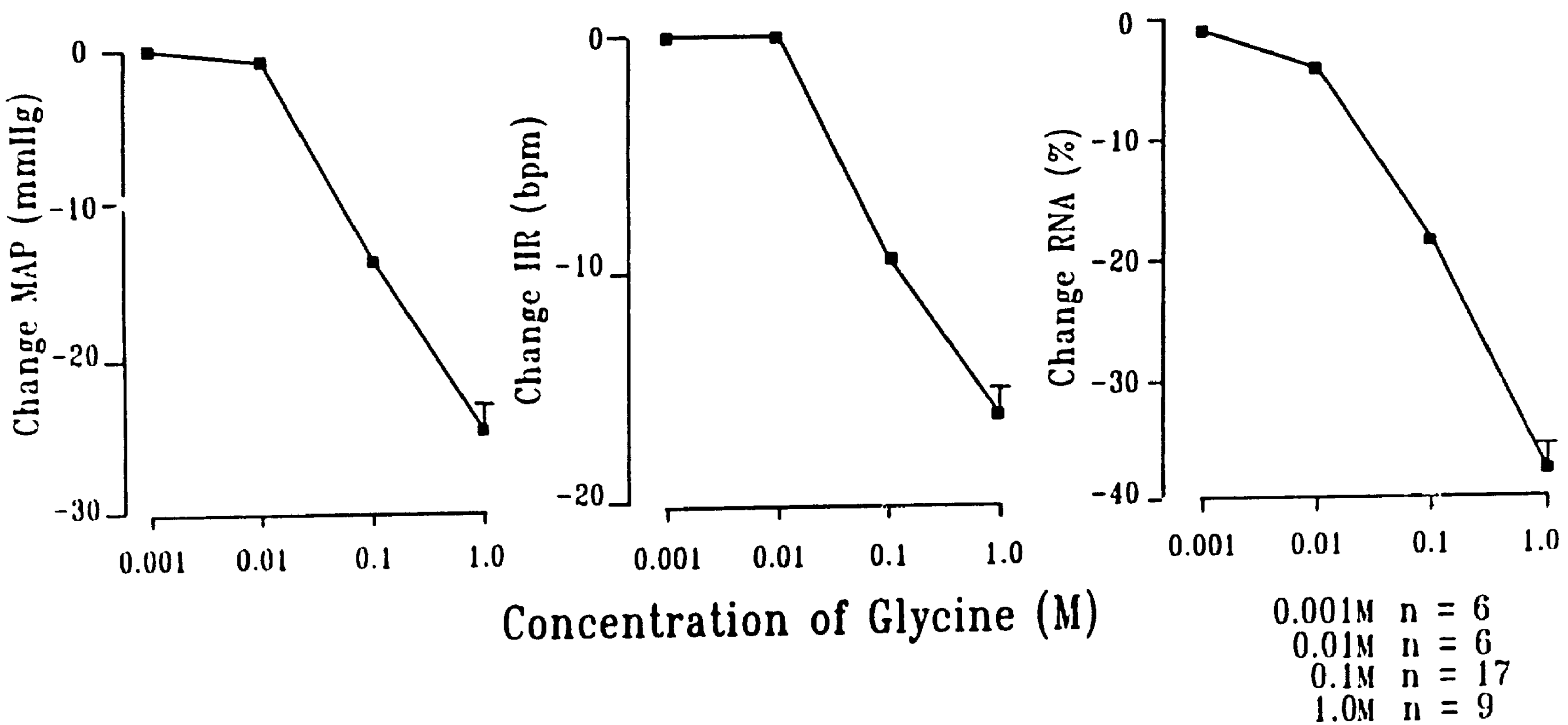
different from decreases in splenic nerve activity of 45 ±4% (from 1.8 to 1.1 μV s; pooled S.E.=0.42; Fig. 17).

A dose-response relationship for glycine was established to determine the most effective dose of glycine to use in this study (Fig. 18). The threshold dose of 0.01M glycine produced small but significant decreases in renal nerve activity and no changes in arterial pressure or heart rate. No significant responses were produced by 0.001M glycine. Since large consistent responses were elicited by 1.0M glycine, this concentration was used for all subsequent experiments.

4.4.3 Microinjections of glycine into the rostral ventrolateral medulla (RVLM).

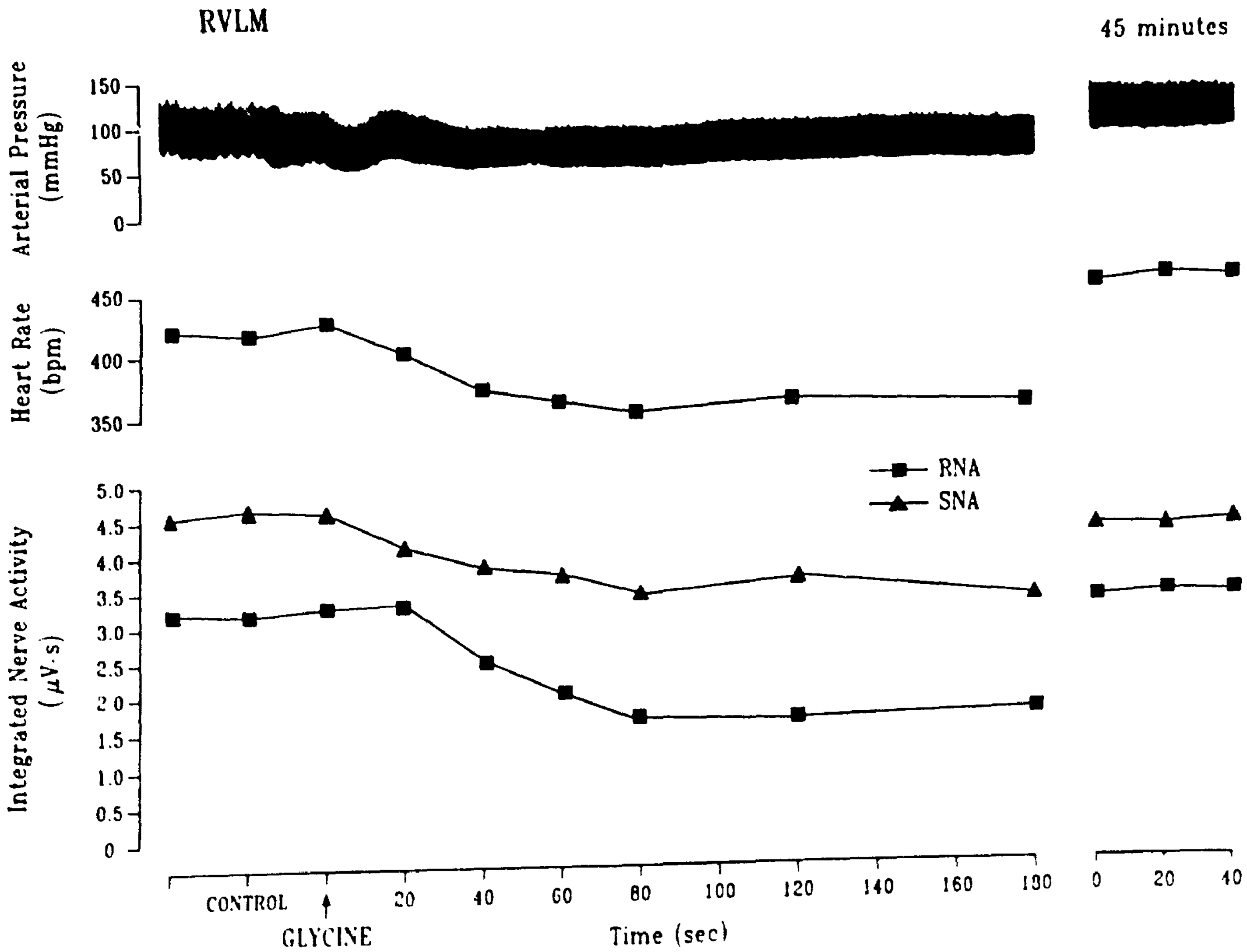
An example of the arterial pressure, heart rate and sympathetic nerve response produced by a unilateral injection of glycine into the RVLM is shown in Fig. 19. In contrast to the cardiovascular and sympathetic responses to PRF blockade, unilateral glycine injections (n=6) into the RVLM produced more prolonged decreases in arterial pressure, heart rate and sympathetic nerve activity which reached a peak response in 107 ±19s and did not return to control values until 41 ±3 min after glycine injection. Mean arterial pressure was significantly reduced by 27 ±4 mmHg (from 97 to 70 mmHg; pooled S.E.=2.9) and heart rate was decreased by 24 ±9 bpm (from 361 to 337 bpm; pooled S.E.=6.7) following RVLM blockade (Fig. 20). In addition, glycine injection into the RVLM caused larger decreases in renal nerve activity (50 ±4%;from 1.5 to 0.84 μV·s; pooled S.E.=1.5) than in splenic nerve activity (31 ±6%;from 1.8 to 1.3 μV·s; pooled S.E.=1.4; Fig. 20). The magnitude of the arterial pressure, heart rate and sympathetic responses elicited by glycine injection into the RVLM were not

Figure 18. The relationship between the concentration of glycine (x-axis) and the mean changes in mean arterial pressure (MAP), heart rate (HR) and renal nerve activity (RNA; y-axes) caused by injections into the pontine reticular formation. Variability is indicated by a pooled standard error calculated from the ANOVA. Numbers of injections at each concentration in nine rats is shown in the inset. Microinjections of 1.0M glycine caused significantly greater decreases in arterial pressure, heart rate and renal nerve activity than did injections of 0.001M, 0.01M or 0.1M glycine. Injections of 0.1M glycine also caused greater decreases in these variables than did injections of the lower two concentrations. Responses to 0.01M glycine did not differ from responses to 0.001M glycine.



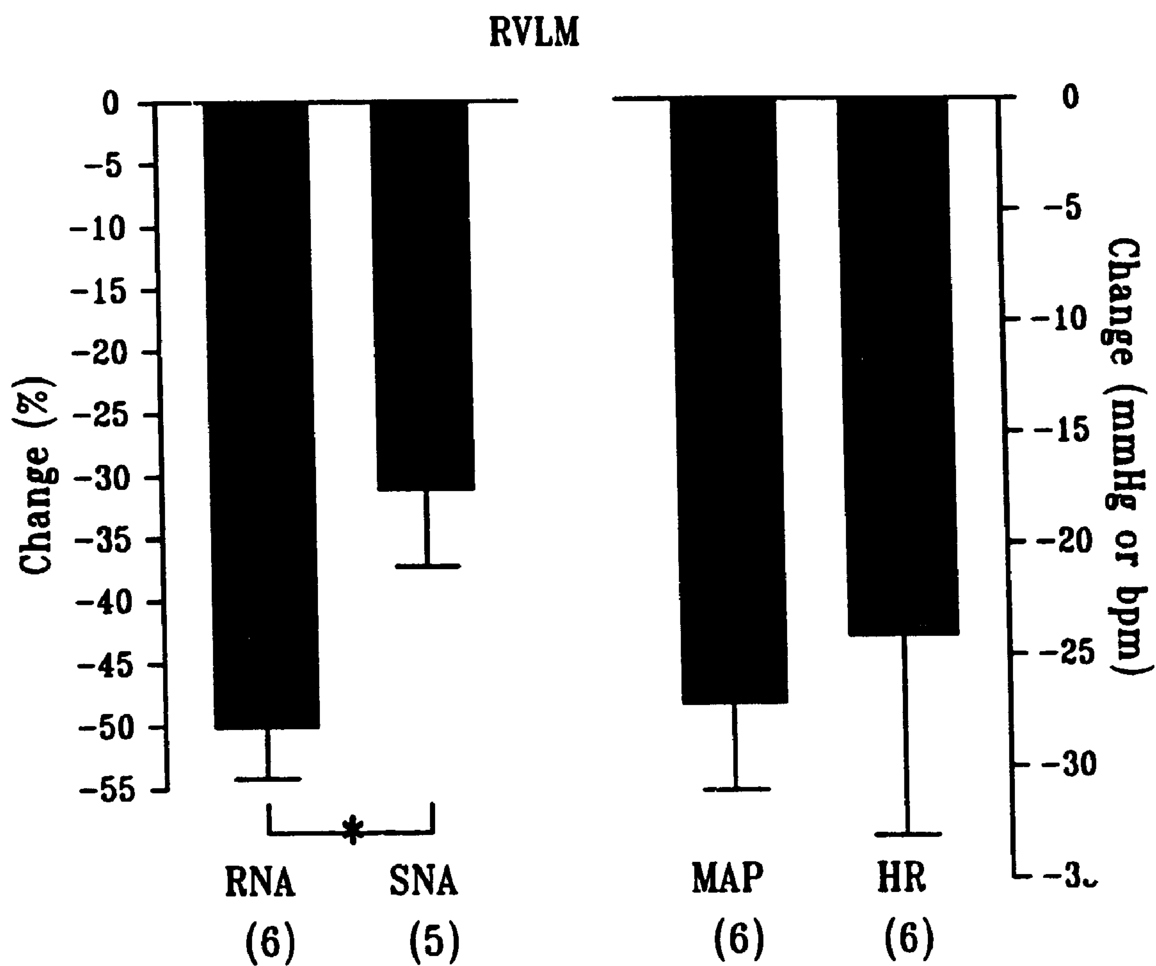
Change MAP (mmHg)
0
-10
-20
-30
0.001
0.01
0.1
1.0
Change HR (bpm)
0
-10
-20
0.001
0.01
0.1
1.0
Change RNA (%)
0
-10
-20
-30
-40
0.001
0.01
0.1
1.0
Concentration of Glycine (M)
0.001M n = 6
0.01M n = 6
0.1M n = 17
1.0M n = 9

Figure 19. Responses to a unilateral injection (65 nl) of glycine into the rostral ventrolateral medulla (RVLM) of one rat. Format is as in **Figure 16**. Glycine injection (arrow) caused large decreases in arterial pressure and heart rate and greater decreases in renal nerve activity than in splenic nerve activity. Arterial pressure, heart rate and sympathetic activity reached a peak response in 80s and did not return to control values until 45min after glycine injection.



RVLM
45 minutes
Arterial Pressure (mmHg)
150
100
50
0
Heart Rate (bpm)
450
400
350
Integrated Nerve Activity (μV·s)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
RNA
SNA
CONTROL
GLYCINE
20
40
60
80
100
120
140
160
180
0
20
40
Time (sec)

Figure 20. Mean responses of mean arterial pressure (MAP) and heart rate (HR; right panels) and renal (RNA) and splenic (SNA) nerve activity (left panels) to unilateral injections of glycine into the rostral ventrolateral medulla (RVLM). Format is as in **Figure 17**. Glycine injections caused significant decreases in arterial pressure, heart rate and sympathetic nerve activity. The decrease in renal nerve discharge was significantly greater than the decrease in splenic nerve discharge (*).



RVLM
Change (%)
0
-5
-10
-15
-20
-25
-30
-35
-40
-45
-50
-55
*
RNA
(6)
SNA
(5)
MAP
(6)
HR
(6)
Change (mmHg or bpm)
-5
-10
-15
-20
-25
-30

different from those caused by glycine injection into the PRF.

4.4.4 Microinjections of NaCl and sucrose into the PRF.

Although the absence of responses to 0.001M glycine demonstrated that the volume of injection caused no response, further controls were done to evaluate possible responses to the osmotic stimulus presented by 1.0M glycine. Seven control injections of NaCl (1.0M) into the PRF had no effects on arterial pressure, heart rate or splenic nerve activity. However, in 4 sites (3 animals) NaCl injections caused a significant decrease in renal nerve activity of 26 ±4% (from 2.1 to 1.5 μV·s; pooled S.E.=1.2; n=4 sites). The mean decrease in renal nerve activity for all 7 sites was 15 ±5% (pooled S.E.=3.2). The same volume of glycine (1.0M) microinjected into the same sites (n=7) reduced arterial pressure by 28 ±5 mmHg (pooled S.E.=3.2), heart rate by 24 ±5 bpm (pooled S.E.=3.3) and renal and splenic nerve activity by 44 ±7% (pooled S.E.= 0.87) and 43 ±4% (pooled S.E.=0.99), respectively. The NaCl injections were the first injections made at each site. Because NaCl at this 1.0M concentration appeared to cause selective renal nerve responses and was not an appropriate control, 2.0M sucrose was injected in additional experiments. These control injections presented the same volume and osmotic stimulus as the 1.0M glycine injections used in the study. Four control injections of sucrose (2.0M) into the PRF failed to produce any changes in arterial pressure, heart rate or sympathetic nerve activity. Microinjection of glycine into the same sites decreased arterial pressure (38 ±4 mmHg; pooled S.E.=4.1), heart rate (26 ±5 bpm; pooled S.E.=3.2) and renal (52 ±10%; pooled S.E.=4.0) and splenic (59 ±9%; pooled S.E.=2.1) nerve discharge. The sucrose

injections were the first injections made at each site.

4.4.5 Microinjections of D,L-homocysteic acid (DLH) into the PRF.

Eighteen injections of DLH were made into sites in the same region of the PRF as previously explored with glycine. DLH injections produced inconsistent changes in arterial pressure, heart rate and sympathetic nerve activity. The cardiovascular and sympathetic responses to these injections are shown in Table 3. After DLH injection into the PRF mean arterial pressure was decreased by 23 ±3 mmHg (n=11 sites) or was increased by 22 ±7 mmHg (n=6 sites) or was not changed (n=1 site). In addition, heart rate was decreased by 16 ±2 bpm (n=10 sites) or was increased by 23 ±6 bpm (n=5 sites) or was not changed (n=3 sites). Finally, renal nerve activity was increased by 52 ±8% (n=14 sites) or was decreased by 35 ±6% (n=4 sites) and splenic nerve activity was increased by 27 ±8% (n=2 sites) or was decreased by 40 ±4% (n=15 sites) or did not change (n=1 site) following DLH injections. Although neither the direction nor the combination of cardiovasculır and sympathetic responses could be predicted by the location of the injection sites, one common response pattern occurred following DLH injections into 11 of 18 sites. DLH injections into these sites caused increases in renal nerve activity and concomitant decreases in splenic nerve activity. Injections into 7 of these sites also caused decreases in systemic arterial pressure. An example of this type of response produced by a unilateral injection of DLH into the PRF is shown in Fig. 21.

4.4.6 Baroreceptor denervation.

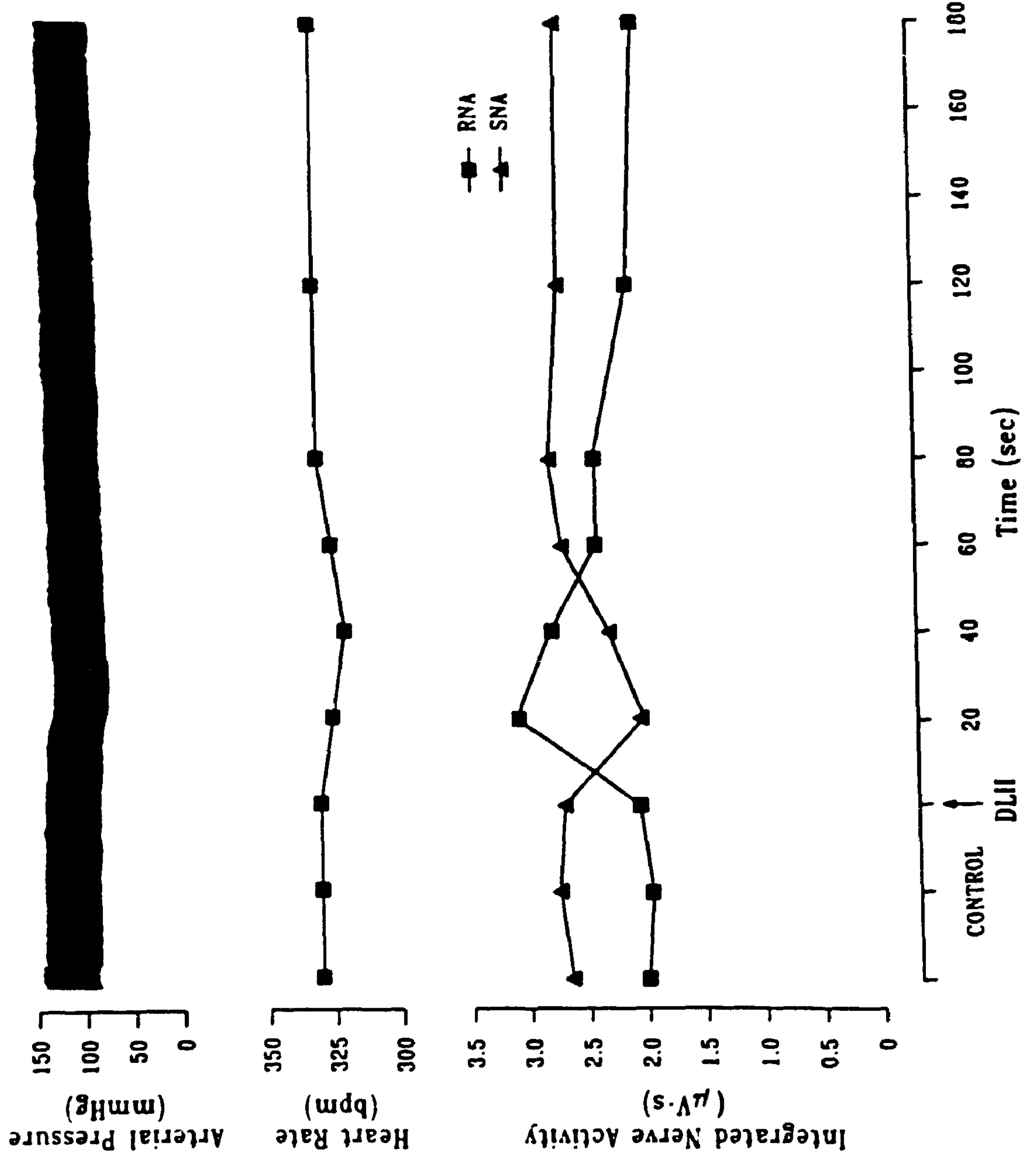
Glycine injections into the PRF produced responses with a very short duration

Table 3. Sympathetic and cardiovascular responses to microinjection of D,L-homocysteic acid into the pontine reticular formation.

	AP	HR	RNA	SNA
rat 910122				
site 1	↓	↓	↑	↓
2	↓	↓	↑	↓
3	-	-	↑	-
4	↑	↑	↑	↑
rat 910107				
site 1	↑	-	↑	↓
2	↓	↑	↓	↓
rat 910109				
site 1	↓	↓	↓	↓
2	↓	↓	↓	↓
3	↓	↓	↑	↓
4	↑	↑	↑	↓
5	↓	↓	↑	↓
6	↑	↑	↑	↑
rat 910129				
site 1	↓	↓	↑	↓
2	↓	↓	↑	↓
3	↓	↓	↑	↓
4	↑	↓	↑	↓
5	↓	-	↓	↓
6	↑	↑	↑	↓

Arrows indicate an increase (↑), decrease (↓) or no change (-) in arterial blood pressure (AP), heart rate (HR) and renal and splenic nerve activity (RNA,SNA).

Figure 21. Responses to a unilateral injection (65 nl) of D,L-homocysteic acid (DLH) into the pontine reticular formation of one rat. Format is as in **Figure 16**. DLH injection (arrow) into this site evoked an increase in renal nerve activity and simultaneous decreases in splenic nerve activity, arterial pressure and heart rate.



Arterial Pressure (mmHg)
150
100
50
0
Heart Rate (bpm)
350
325
300
Integrated Nerve Activity (μV·s)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
RNA
SNA
CONTROL
DLII
20
40
60
80
100
120
140
160
180
Time (sec)

while long-lasting responses were observed after injections into the RVLM. The rapid recovery of PRF responses may reflect the efficiency of baroreceptors to compensate for the large fall in arterial pressure which occurs after glycine injection. To test the involvement of the baroreceptor reflex in this response, glycine was injected into the PRF of sino-aortic denervated rats. Unilateral glycine injections (n=7) into the PRF of rats with sino-aortic denervation caused decreases in arterial pressure (28 ±3 mmHg;from 113 to 85 mmHg; pooled S.E.=2.6), heart rate (20 ±2 bpm; from 397 to 377 bpm; pooled S.E.=1.7) and renal (57 ±5%; pooled S.E.=3.2) and splenic (61 ±5%; pooled S.E.=1.0) nerve activity. As in intact rats, the responses of renal and splenic nerves to glycine injections were not different. The cardiovascular and sympathetic responses in these animals reached a peak response in 24 ±2s and returned to control values as rapidly as those in intact rats (within 132 ±27s). The magnitude of the arterial pressure, heart rate and sympathetic nerve responses to glycine injection in denervated rats were not different from those in intact rats.

4.4.7 Bilateral microinjections of glycine into the PRF.

To investigate the possibility that inhibition of activity of PRF neurons following a unilateral glycine injection may be opposed by discharge of contralateral PRF neurons, bilateral glycine injections were made into the PRF. These bilateral injections (n=3) produced decreases in heart rate and sympathetic nerve activity that were not different from those produced by unilateral glycine injection. Bilateral injections decreased heart rate by 17 ±3 bpm (from 373 to 357 bpm; pooled S.E.=1.9) and caused similar decreases in renal (49 ±6%; pooled S.E.=1.4) and splenic (54 ±4%; pooled S.E.=1.3)

nerve activity. However, bilateral PRF blockade caused larger decreases in arterial pressure of 47 ±4 mmHg (from 115 to 68 mmHg; pooled S.E.=3.2) than those caused by unilateral blockade. Responses following bilateral glycine injections reached a peak in 30 ±8s and returned to control values as rapidly as unilateral responses (within 87 ±14s).

4.4.8 Microinjections of muscimol into the PRF.

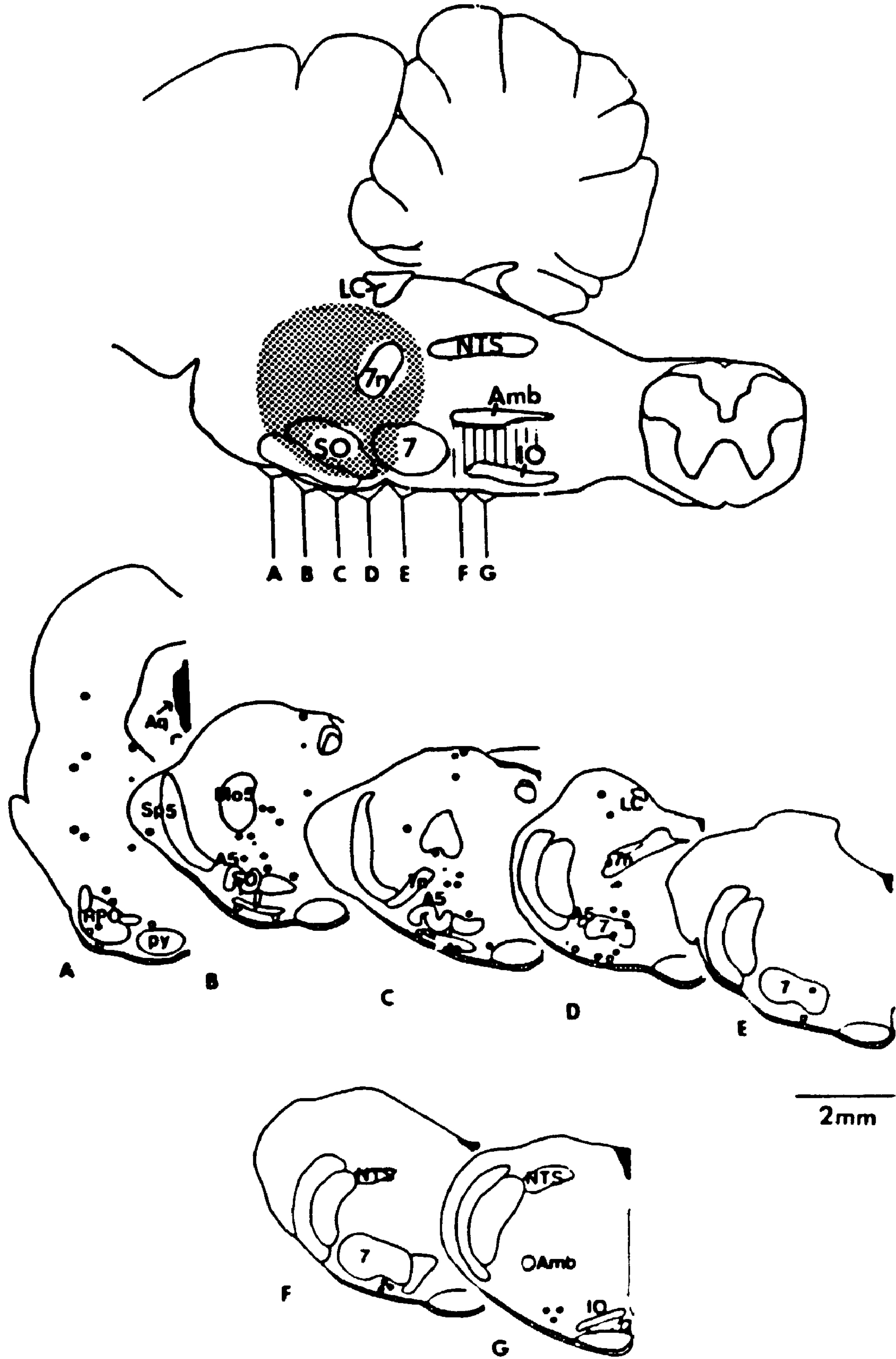
Finally, PRF responses may have been transient because glycine has a short action on neurons in this area. To investigate this possibility the long-lasting $GABA_A$ agonist muscimol was injected unilaterally into the PRF. These injections (n=4) reduced arterial pressure by 36 ±7 mmHg (from 120 to 84 mmHg; pooled S.E.=5.3) and heart rate by 16 ±5 bpm (from 363 to 347 bpm; pooled S.E.=3.4) and decreased ongoing discharge of renal nerves by 55 ±15% (pooled S.E.=2.0) and splenic nerves by 47 ±10% (pooled S.E.=1.7). As with glycine injections, responses of renal and splenic nerves to muscimol injection were not different from each other. Responses following muscimol injections reached a maximum decrease in 18 ±2s and returned to control values within 95 ±25s. The magnitude of the arterial pressure, heart rate and sympathetic responses to unilateral injection of muscimol were not different from those caused by unilateral injection of glycine.

4.4.9 Histological verification of injection sites.

The locations of the histologically verified injection sites in the PRF, plotted on modified drawings (Paxinos & Watson, 1986) of transverse sections of the rat brainstem are shown in Fig. 22. Sites in which glycine produced decreases in mean

arterial pressure, heart rate and renal and splenic nerve activity were found in a region extending caudally from the border of the midbrain (0.70 mm rostral to interaural zero) to the middle region of the facial nucleus (1.80 caudal to interaural zero). Sites in which glycine did not elicit cardiovascular and sympathetic responses are also plotted (n=27) and these sites were found at the rostral boundary, on the ventral surface or more than 3mm lateral to the midline. No viscerotopic organization of sites sensitive to either renal or splenic nerves was found in the PRF. Sites into which muscimol or DLH were injected are not plotted in Fig. 22. However, all of these sites were located within the same region of the PRF as were the glycine injection sites. In addition 4 cerebellar sites in which glycine failed to produce responses are not plotted in Fig. 22. Unresponsive sites could be as close as 500 μm to responsive sites indicating the effective diffusion of the glycine injections was not more than this distance.

Figure 22. Pontine and medullary sites of glycine injection. Top panel: stylized drawing of a sagittal section of rat brainstem. Stippled area indicates the pontine reticular formation (PRF) and vertical lines indicate the region of the rostral ventrolateral medulla (RVLM). A, B, C, D, E, F, G refer to the rostrocaudal regions represented in the transverse sections shown in the bottom panel. Bottom panel: large and small filled circles represent injection sites in which glycine produced changes in sympathetic nerve activity ≥30% or ⟨30% respectively. Open circles represent injection sites in which glycine did not elicit a change in sympathetic nerve activity. LC (locus coeruleus); NTS (nucleus tractus solitaris); Amb (nucleus ambiguus); 7n (facial nerve); 7 (facial nucleus); A5 (region of the A5 cell group); SO (superior olive); IO (inferior olive); Aq (aqueduct); py (pyramidal tract); Sp5 (spinal trigeminal nucleus); RPO (rostral periolivary region); Mo5 (motor trigeminal nucleus).



LC
NTS
7n
Amb
SO
7
IO
A B C D E F G
Aq
Sp5
Mo5
A5
SO
RPO
py
LC
7n
A5
7
A
B
C
D
E
2mm
NTS
NTS
7
Amb
IO
F
G

4.5 Discussion

The most important finding of this study was that inhibition of discharge of neurons in the pontine reticular formation (PRF) evoked large decreases in arterial pressure and activity of renal and splenic sympathetic nerves and smaller changes in heart rate. These results identify a source of cardiovascular control in this region that provides tonic excitatory drive to sympathetic nerves. Tonic vasomotor control from the pons has not been reported previously. Groups of neurons involved in tonic control of sympathetic activity and arterial pressure stretched rostrally to the border of the midbrain (the level of the aqueduct) and caudally to the middle region of the facial nucleus and were rostral to the well-defined rostral ventrolateral medulla (RVLM). Cardiovascular and sympathetic responses could not be elicited from sites on the ventral surface but appeared to originate primarily from a disperse region spanning the rostrocaudal reticular "core" of the pons.

The cardiovascular and sympathetic responses caused by inhibition of neurons in the PRF differed from those caused by RVLM blockade. Microinjections of glycine into the RVLM caused greater reductions in renal than in splenic nerve activity. This is consistent with many experiments in rats and cats which have shown that the RVLM does not produce equal activation of all sympathetic nerves (Stein *et al.*, 1989; Yardley *et al.*, 1989a; Hayes & Weaver, 1990; Beluli & Weaver, 1991a). In contrast, inhibition of tonic activity of PRF neurons caused equal responses in renal and splenic nerves. It is possible that neurons in the PRF generate sympathetic drive equally distributed to renal and splenic nerves and that differential organization of sympathetic

outflow to these vascular beds is intrinsic to neuronal organization elsewhere.

Furthermore, responses of arterial pressure, heart rate and sympathetic nerve discharge were long-lasting following RVLM blockade while glycine injection into the PRF produced short duration responses. After PRF blockade some mechanism appeared to act immediately to compensate for the large decreases in arterial pressure and nerve activity. The rapid recovery of PRF responses was not the result of reflex increases in sympathetic discharge due to baroreceptor unloading since after PRF blockade, arterial pressure and sympathetic nerve activity recovered as rapidly in baroreceptor-denervated animals as in intact animals.

The brief time course of PRF responses was also not due to a short action of glycine in this area. This study and previous work (Hayes & Weaver, 1990) have shown that glycine inhibits the activity of RVLM neurons for at least 40 min. In addition, injections of the long-lasting $GABA_A$ agonist muscimol into the PRF produced cardiovascular and sympathetic responses which were equivalent in magnitude and as transient as those caused by glycine injection. Therefore, an action on PRF neurons specific to glycine or glycine receptors is not a probable explanation for the brief character of the PRF response.

An additional hypothesis is that sympathetic activity is generated by a network of neurons extending throughout the PRF. The loss of discharge of some of these neurons would have transient effects as discharge from the remainder of the network could quickly compensate to restore sympathetic drive. Although cardiovascular and sympathetic responses following bilateral injections of glycine into the PRF were as

brief as those after unilateral injections, we cannot exclude the possibility that network neurons not inhibited by glycine may still have been capable of generating sympathetic discharge effectively. No attempt was made to inhibit greater areas of the PRF because larger injection volumes may have resulted in diffusion of glycine into the RVLM.

Finally, increased activity of neurons in the spinal cord or in brain regions outside of the pons may have compensated for the loss of sympathetic drive once activity of PRF neurons had been inhibited. One area that is likely to contribute to the rapid recovery of sympathetic activity is the RVLM since activity of RVLM neurons may be generated both by synaptic inputs (Barman & Gebber, 1987; Gebber & Barman, 1988) or independently due to intrinsic pacemaker properties in some of these neurons (Sun *et al.*, 1988a; 1988b; 1988c).

Microinjection of excitatory amino acids into the PRF illustrated another important difference between sympathetic and cardiovascular control by pontine neurons and control by the RVLM. Whereas excitation of RVLM neurons with D,L-homocysteic acid (DLH) produces consistent increases in arterial pressure, heart rate and sympathetic nerve activity (Beluli & Weaver, 1991a), responses to DLH injections into the PRF were inconsistent. Since DLH injections most commonly caused increases in renal and decreases in splenic nerve activity, the inhibitory responses to DLH were not caused by depolarization blockade (Lipski, Bellingham, West, & Pilowsky, 1988). In addition, this variety of responses could not have been due entirely to the large volume of DLH injected at each PRF site because consistent responses were caused by

injection of the same volume of glycine. Instead, the PRF may contain a functionally heterogenous population of neurons that can be activated to influence sympathetic outflow. However, within this population, tonically active neurons involved in providing basal vasomotor activity appear to be a homogenous neuronal group.

The responses of renal nerves to microinjection of NaCl (1.0M) into the PRF were unexpected. Renal nerve responses were not caused by tissue damage or by activation of osmoreceptors since injection of an equivalently hypertonic solution of sucrose (2.0M) had no effect on renal nerve discharge. Instead, responses of renal nerves may have involved a sodium-specific mechanism. At least one other specific brainstem area, the nucleus tractus solitaris is sensitive to hypertonic concentrations of NaCl and injections of NaCl (154mM) into this area cause hypotension and bradycardia (Lukovic & de Jong, 1990). However, to our knowledge, there have been no studies of sodium sensitive neurons within the PRF.

In initial experiments (Hayes, Beluli & Weaver, 1990b) we explored the pons in 5 rats anaesthetized with urethane, a commonly used anaesthetic in experiments studying sympathetic control of the cardiovascular system. However, glycine injections (n=7) into the PRF of these animals caused smaller decreases in arterial pressure (-17 ±7 mmHg), heart rate (-9 ±3 bpm) and renal (-17 ±8%) and splenic (-19 ±9%) sympathetic nerve activity than were caused by glycine injections into the PRF of Saffan-anaesthetized rats. Even though cardiovascular and sympathetic responses elicited from the RVLM were similar in rats anaesthetized with either urethane (Hayes & Weaver, 1990) or Saffan, these anaesthetics may have selective effects on the

activity of neurons outside of the RVLM that are generating sympathetic discharge. For example, pentobarbital anaesthesia reduces the spontaneous discharge rate and the neuronal responsiveness of medullary reticular neurons (Morrow & Casey, 1983), a neuronal network also thought to play an important role in generating vasomotor tone (Barman & Gebber, 1987; Gebber & Barman, 1988). In addition, although few neurons in the rostral medullary reticular formation have discharges correlated with sympathetic nerve activity in cats anaesthetized with barbiturate, the discharges of 50% of these neurons are correlated with sympathetic activity in cats anaesthetized with chloralose (Kenney, Barman & Gebber, 1989). Although not tested, urethane may depress the activity of reticular neurons to a greater extent than do other anaesthetics causing maintenance of resting arterial pressure in these animals to become more dependent upon discharge from neurons in other brain areas such as the RVLM. Furthermore, although some experiments including the previous studies have shown that baroreceptor stimulation causes unequal decreases in renal and splenic nerve activity (Meckler & Weaver, 1988; Hayes & Weaver, 1990), equal responses of renal and splenic nerves were observed in the present study. Since others have reported selective effects of anaesthetics on baro- and chemoreceptor reflex responses (Ishikawa, Kallman & Sagawa, 1984; Dorward, Burke, Janig & Cassell, 1987), differences in the sympathetic responses to baroreceptor stimulation may be related to the different anaesthetics used. These observations suggest that the type of anaesthesia has dramatic effects on the organization of neuronal networks responsible for resting and reflex sympathetic activity. Although Saffan is no longer available, recent studies

in our laboratory have been done using propofol (Diprivan, I.C.I. Pharma, Mississauga, Canada), an intravenously administered anaesthetic commonly used in human surgery (Sebel & Lowdon, 1989). Rats under this anaesthesia also demonstrate large responses to blockade of the PRF or RVLM by glycine (Krassioukov, Gelb & Weaver, 1992).

In conclusion, a previously unknown source of tonic cardiovascular control in the reticular formation of the pons has been identified in this investigation. Tonic activity of neurons within the PRF makes a contribution to ongoing activity of sympathetic nerves supporting arterial pressure and heart rate. This evidence demonstrates that regions outside the classical vasomotor centre may be important in generation of tonic sympathetic discharge and supports the concept that generation of this drive is the collective responsibility of a network of brainstem neurons.

Chapter 5: Pontine reticular neurons provide tonic excitation to neurons in the rostral ventrolateral medulla in rats.

American Journal of Physiology, 1993, In press.

5.1 Introduction

Sympathetic nerve activity supporting arterial pressure is generated by groups of neurons located throughout the brainstem. The best known area involved in tonic cardiovascular control is located in the rostral portion of the ventrolateral medulla (RVLM). Neurons in the RVLM send axonal projections directly to sympathetic preganglionic neurons located in the spinal cord (Morrison *et al.*, 1988; Sun *et al.*, 1988a; Ruggiero *et al.*, 1989). Since destruction of this discrete group of RVLM neurons (Granata *et al.*, 1985a; 1986) or inhibition of their activity (Pilowsky *et al.*, 1985; Stein *et al.*, 1989; Yardley *et al.*, 1989a; Hayes & Weaver, 1990; Beluli & Weaver, 1991b) produces large decreases in arterial pressure, heart rate and sympathetic nerve discharge, the activity of these neurons is thought to be important for maintaining vasomotor tone. However, neurons located in the pontine reticular formation (PRF) also make a contribution to ongoing activity of sympathetic nerves. In rats, inhibition of activity of PRF neurons by microinjections of the inhibitory amino acid glycine or the $GABA_A$ agonist muscimol produces large, short-lasting decreases in arterial pressure, heart rate and sympathetic nerve activity (Hayes & Weaver, 1992; Krassioukov *et al.*, 1992; Krassioukov & Weaver, 1992). The neural pathways mediating these responses from the PRF are unknown.

Although both the PRF and the RVLM contribute to basal sympathetic nerve discharge, the exact region(s) of origin of sympathetic activity remain(s) to be determined. Sun *et al.*, (1988b) have demonstrated in rats that some neurons in the RVLM develop pacemaker-like activity after intracisternal administration of the glutamate receptor antagonist kynurenate and that the regular discharge pattern of RVLM neurons in slices arises from typical pacemaker potentials (Sun *et al.*, 1988a). Although it is possible that these neurons are responsible for generating sympathetic nerve discharge, it cannot be excluded that RVLM neurons may also receive a tonic excitatory drive from neurons located in other brainstem regions (Barman & Gebber, 1987; Gebber & Barman, 1988). The present study was done to determine if ongoing activity of neurons in the PRF is a source of tonic excitatory drive to sympathoexcitatory neurons located in the RVLM.

5.2 Methods

Surgical procedures

Fourteen male Wistar rats (250-420 g; Charles River, Canada) were anaesthetized initially with sodium pentobarbital (40 mg/kg, i.p., M.T.C. Pharmaceutical, Canada) and a tracheal tube was inserted. Both jugular veins and the right femoral artery were cannulated for delivery of solutions and drugs and for monitoring arterial blood pressure, respectively. Arterial blood samples (~100μl) were withdrawn periodically for analysis of pH, pO_2 and pCO_2 and deviations from normal were corrected by i.v. administration of sodium bicarbonate or by adjusting respiratory rate and/or tidal volume. Body temperature was maintained at 37°C with a heating pad. An infusion of the steroid anaesthetic Saffan (alphaxalone 9mg/ml and alphadolone 3mg/ml, Glaxovet, Glaxo Canada Ltd., Canada) via one venous cannula (0.4-1.0 ml/hr) was begun as effects of the pentobarbital disappeared and adjustments in the rate of infusion were made as needed to maintain a constant plane of anaesthesia. The animal's plane of anaesthesia was assessed by examining palpebral and withdrawal reflexes. Physiological saline was infused slowly ($\leq$0.6 ml/hr) through the other venous cannula to compensate for fluid loss. The animals were artificially respired with oxygen-enriched air and paralysed with gallamine triethiodide (20 mg/kg initially, followed by doses of 10 mg/kg as needed, Rhone-Poulenc, Canada). Before each supplemental dose of gallamine was given, the animal's plane of anaesthesia was assessed. The rats were placed in a stereotaxic frame (David Kopf Instruments, USA) with the bite bar 20 mm below the interaural line. The dorsal neck muscles were

retracted and the interparietal and occipital bones were removed exposing the cerebellum overlying the pons and medulla. The atlanto-occipital membrane was cut and the dura was removed. The surface of the brain was kept moist with saline-soaked gauze. The electrocardiogram (ECG) was recorded with pin electrodes inserted into muscle. Before and throughout the experiment, the muscarinic antagonist atropine (1 mg/kg, bolus i.v., Sigma Chemical Company, USA) was given at 2 hr intervals to ensure that any observed changes in heart rate were due to changes in cardiac sympathetic nerve activity.

Recordings from renal nerves

The left kidney was exposed by a left flank incision and small postganglionic nerve bundles were dissected from the renal artery and severed. The central ends were placed on small stainless-steel bipolar electrodes and covered with dental impression medium (Perfourm, Cutter Dental, USA) to isolate the nerve and the electrode from the surrounding tissue and to prevent dehydration. Multifibre discharge was amplified at a bandwidth of 1 Hz-1 kHz to display the synchronized discharge of sympathetic nerve discharge in the form of 2-6 Hz slow waves. The amplified signals and the ECG were monitored on an oscilloscope, recorded on magnetic tape (Racal, model 7DS, UK) and displayed with arterial pressure and heart rate on a Grass Instruments polygraph (Grass Instrument Company, USA). A pneumothorax was made to eliminate artifacts in the neural recordings caused by respiratory movements. To ensure that recordings were from sympathetic nerves containing vasomotor fibres, arterial baroreceptors were stimulated by increasing arterial pressure with a bolus i.v.

injection of 1-3 μg phenylephrine (Neo-Synephrine, Sterling, Canada) to elicit decreases in heart rate and renal nerve activity. In all 14 animals, an increase in arterial pressure from 106 ±4 to 168 ±5 mmHg decreased heart rate by 19 ±3 bpm (from 328 ±8 bpm) and inhibited ongoing discharge of renal nerves by 58 ±7% (from 2.2 ±0.4 to 1.1 ±0.3 μV·s). At the end of the experiment, background electrical noise was recorded for 2 min after the animals had been given an overdose of urethane anaesthetic. Neural discharge recorded on magnetic tape was digitized, rectified and integrated cumulatively during 10 s periods. After subtracting background electrical noise, integrated nerve activity was expressed as μV·s.

Recordings from RVLM units

Activity from spontaneously firing units in the rostral ventrolateral medulla (RVLM) was recorded extracellularly with glass micropipettes filled with 0.5 M sodium acetate and 2% Pontamine sky blue (4-10 MΩ impedance at 1 kHz). The micropipettes were advanced through the cerebellum using a hydraulic microdrive (model MO8, Narishige, Japan) and the tips positioned in the RVLM (stereotaxic coordinates: 2.5-2.9 mm rostral to obex, 1.7-1.9 mm lateral to midline and within 500 μm of the ventral surface; Paxinos & Watson, 1986). Electrical activity was amplified by a preamplifier (Dagan 2400) using bandpass filters set at 0.3-3 kHz and displayed on an oscilloscope. Unit activity, ECG, renal sympathetic nerve activity and event markers were acquired by an AST (model 286-AT) computer using RC Electronics Computerscope (RC Electronics Inc., U.S.A.) for on-line analysis during the experiment and were also recorded on magnetic tape for off-line analysis. The

recording sites in the RVLM were marked with iontophoretic deposits of Pontamine blue (15 μA of cathodal current for 8 min).

Microinjection procedures

Glass micropipettes were pulled to a tip size of about 40 μm and filled with a 1.0M solution of the inhibitory amino acid glycine (BDH Chemicals, Canada) in distilled water (pH=7.4). The pipettes were inclined 10° from the vertical in the sagittal plane with the tip pointing caudally and the tips were positioned within the PRF (stereotaxic coordinates: 5.0-5.5 mm rostral to obex, 1.8 mm lateral to midline and 8.0 mm below the dorsal surface of the brain; Paxinos & Watson, 1986). To inhibit the ongoing activity of neurons in the pontine reticular formation (PRF), glycine was microinjected unilaterally into the PRF using a picospritzer (General Valve Corporation, USA). Injection volumes (45-70 nl) were determined by the displacement of the meniscus at the air-liquid interface in the pipette observed through a microscope containing an ocular micrometer. A few drops of India ink were added to 10 ml of the glycine solution to mark the injection site in the brain. Control injections of a solution of sucrose (2.0M, 50nl) into the PRF did not produce any changes in arterial pressure, heart rate or sympathetic nerve activity as demonstrated in a previous study (Hayes & Weaver, 1992).

Experimental protocol

At the start of each experiment, a site within the PRF from which decreases in arterial pressure, heart rate and renal nerve activity could be elicited by unilateral injection of glycine (57 ±3nl) was identified. After placement of this microinjection

electrode, the ipsilateral RVLM was searched for spontaneously firing neurons. Once a stable recording (signal-to-noise ratio >2) was obtained, each unit was characterized by means of 3 tests. First, the change in the discharge rate of each RVLM unit to an increase in arterial pressure caused by a bolus i.v. injection of phenylephrine (1-3 μg) was recorded. Second, the correlation of spontaneous unit activity to the cardiac cycle was tested by constructing a post R-wave (of the ECG) triggered histogram. Units that were inhibited by baroreceptor activation and that were synchronized to the cardiac cycle were regarded as "cardiovascular" neurons (Brown & Guyenet, 1985; Agarwal *et al.*, 1990). Third, RVLM neuronal spike-triggered averages of renal nerve discharge were constructed to determine if the RVLM unit activity was temporally correlated to the 2-6 Hz rhythmic discharge of sympathetic nerves (Barman & Gebber, 1981; Gebber & Barman, 1988). "Dummy" averages were obtained by triggering averages of sympathetic discharge with a random pulse series generated by a stimulator (Grass Instrument Company, USA). The dummy averages were constructed using pulses of the same mean firing frequency as the unit and the same number of sweeps as for the unit-triggered average. Neurons were considered to have sympathetic-related activity when the amplitude of a peak in the spike-triggered averaged sympathetic activity was at least 3 times larger than that of the largest oscillations of the dummy average. Finally, to determine the predominant frequency components in renal nerve activity, electrical discharge of renal nerves was passed through a 50-Hz low-pass filter and subjected to power density spectral analysis. The filtering was done to eliminate aliasing of high-frequency components of the discharge

as low-frequency components (Malmstadt, Enke & Crouch, 1981). Software from RC Electronics (RC Electronics Inc., U.S.A.) was used to discriminate unit activity from background noise, for construction of post R-wave histograms, for unit-triggered averages of renal nerve activity and for power density spectral analysis.

While recording the spontaneous activity of RVLM cardiovascular neurons, the tonic activity of PRF neurons was inhibited by microinjection of glycine (54 ±2nl). A minimum interval of 65 min between injections of glycine into the pons was allowed because it has been shown in a previous study that 2 consecutive glycine injections into the same site could elicit equal responses if separated by this interval (Hayes & Weaver, 1990). Continuous rate-meter histograms of RVLM unit discharge (RC Electronics Inc., U.S.A.) were constructed and spontaneous unit activity was recorded from 1 min before to 5 min after glycine injection. To evaluate the specificity of pontine influences on RVLM units, the spontaneous activity of neurons in the region of the RVLM that were not affected by activation of baroreceptors and not synchronized to the cardiac cycle was also recorded and the effect of glycine microinjection into the PRF on the ongoing discharge of these "non-cardiovascular" units was studied.

Histology

The brainstems were removed and stored in a solution of 10% formalin in saline. Transverse sections of 50 μm were cut on a cryostat, stained with Neutral Red dye and viewed using a Leitz microscope (Wild-Leitz, Canada). The sites of microinjection in the PRF were identified by India ink deposits and the recording sites in the region of

the RVLM were identified by deposits of Pontamine sky blue. Microinjection and recording sites were mapped on drawings of transverse sections of the rat medulla (Paxinos & Watson, 1986).

Statistical analysis

Arterial pressure, heart rate and renal nerve responses for individual PRF injections were determined using 95% confidence intervals. A one-way analysis of variance (ANOVA) with repeated measures was used to determine statistical changes in mean neural discharge, heart rate and arterial pressure after baroreceptor activation and glycine injection into the PRF. Differences were considered significant when $p<0.05$ and variability was expressed as the pooled standard error derived from ANOVA or as the standard error of the mean (Snedecor & Cochran, 1980). Responses of individual units to baroreceptor activation and to PRF blockade were also analyzed using 95% confidence intervals where possible. As some cardiovascular units displayed spontaneous activity which included occasional 2s silent intervals during the control period, it was necessary to distinguish inhibition of unit firing due to glycine injection into the PRF from spontaneous silent intervals. Because after PRF blockade, renal nerve activity was never inhibited for periods shorter than 20s, inhibition of unit activity which had a duration shorter than 20s was not considered to be a response to glycine injection.

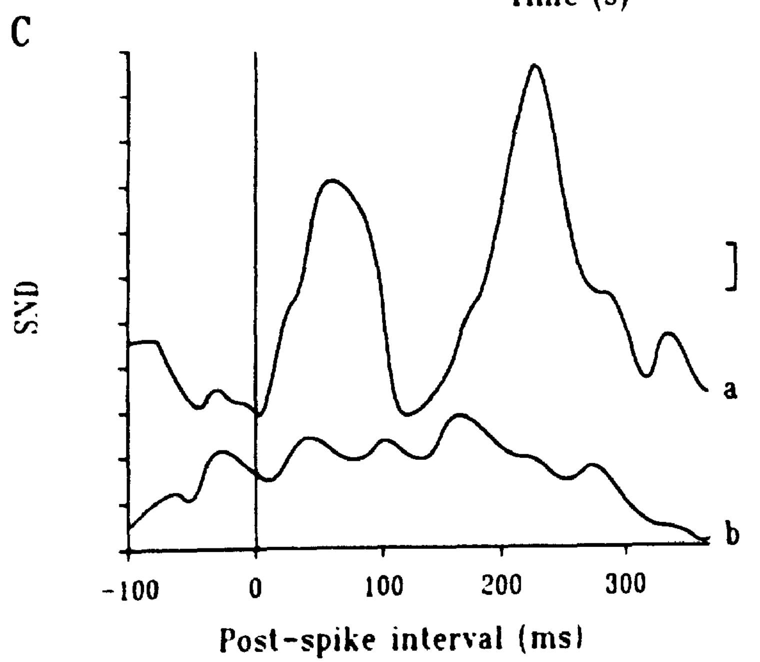
5.3 Results

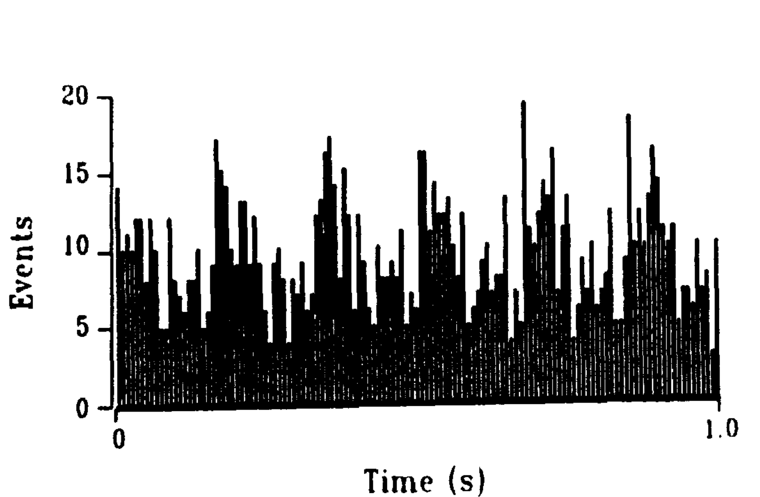
Spontaneous activity was recorded from 24 units located in the region of the RVLM. Of these 24 units, 15 units were considered to be cardiovascular because their spontaneous activity was inhibited by an increase in arterial pressure and because they displayed a clear cardiac cycle rhythmicity when systolic pressure was greater than 120 mmHg. Spike-triggered averages of renal nerve discharge revealed that the activity of 7 of these cardiovascular units was also correlated with renal nerve activity while the activity of the remaining 8 cardiovascular units was not correlated. An example of a cardiovascular unit that was silenced by baroreceptor activation and that displayed activity correlated with the cardiac cycle and renal nerve discharge is shown in Fig. 23. The control firing rate of the 15 cardiovascular units ranged from 1.6 to 4.7 spikes/s and the mean firing rate was 2.7 ±0.2 spikes/s.

Unilateral glycine injections (57 ±3nl) into the PRF ipsilateral to the recording site produced significant decreases in arterial pressure, heart rate and renal nerve activity that began within 5-10 seconds, reached a maximum decrease in 10-20s and returned to control values in 25-120s. Mean arterial pressure was decreased by 28 ±5 mmHg (from 123 to 95 mmHg; pooled S.E.=2.8), heart rate was decreased by 23 ±3 bpm (from 359 to 336 bpm; pooled S.E.=1.7) and renal nerve activity was decreased by 42 ±7% (from 1.6 to 1.0 μV·s; pooled S.E.=1.2) following glycine injection. An example of the renal nerve, arterial pressure and heart rate responses produced by a unilateral injection of glycine into the PRF is shown in Fig. 24A.

Glycine injection into the PRF inhibited the spontaneous activity of 10

Figure 23. Identification of a cardiovascular unit in the RVLM. A. effect of phenylephrine-induced increases in arterial pressure (PE) on spontaneous unit activity (rate-meter histogram; bin width 2.5ms). B. post R-wave histogram of unit activity shows a cardiac rhythm (1746 sweeps). C. spike-triggered (trace a) and "dummy" (trace b) averages of renal nerve activity. (751 sweeps; bin width 1ms; vertical calibration 10μV). The response of this unit to PRF blockade is shown in **Figure 24**.


A
unit activity
spikes/bin
0
2
4
6
8
10
PE
20
40
Time (s)


B
Events
0
5
10
15
20
0
1.0
Time (s)

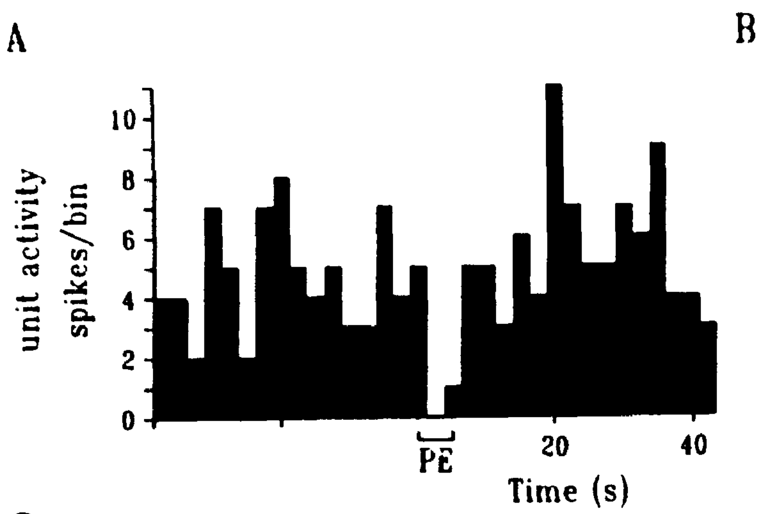
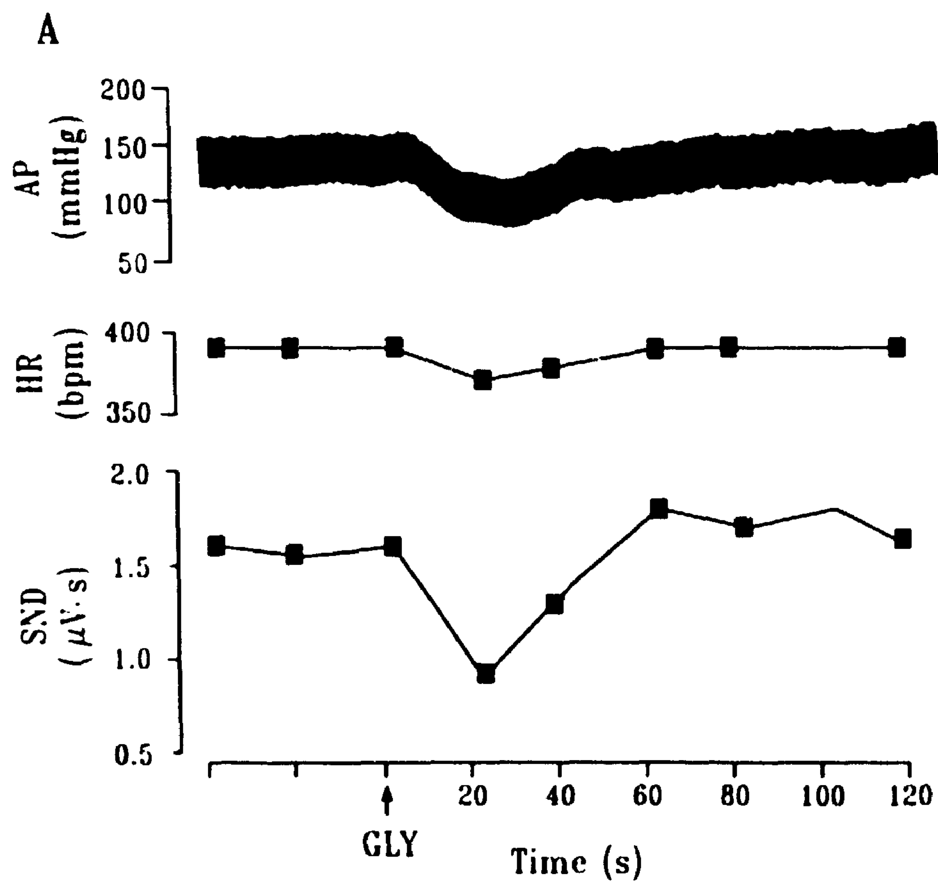
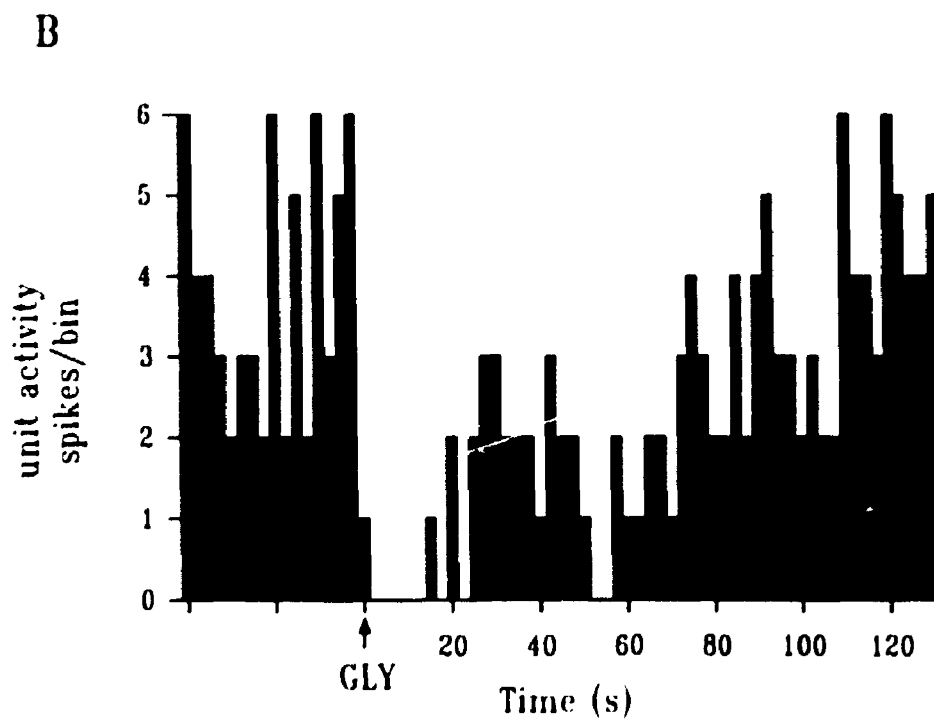

C
SND
a
b
-100
0
100
200
300
Post-spike interval (ms)

Figure 24. Responses to a unilateral microinjection (53nl) of glycine (1.0M) into the pontine reticular formation of a rat. A: record of systemic arterial blood pressure (AP), heart rate (HR) and integrated renal nerve activity (SND). B: rate-meter histogram of spontaneous discharge of a unit located in the RVLM (bin width 2.5ms); this unit met our criteria to be considered cardiovascular as shown in **Figure 23**. Time is indicated on the x-axis beneath these panels. Injection of glycine (arrow) elicited decreases in arterial pressure, heart rate and renal nerve activity and silenced the ongoing activity of this cardiovascular unit. Arterial pressure, heart rate, renal nerve activity and unit activity recovered to control levels within 80s after glycine injection.


A
AP (mmHg)
200
150
100
50
HR (bpm)
400
350
SND (μV·s)
2.0
1.5
1.0
0.5
20
40
60
80
100
120
GLY
Time (s)

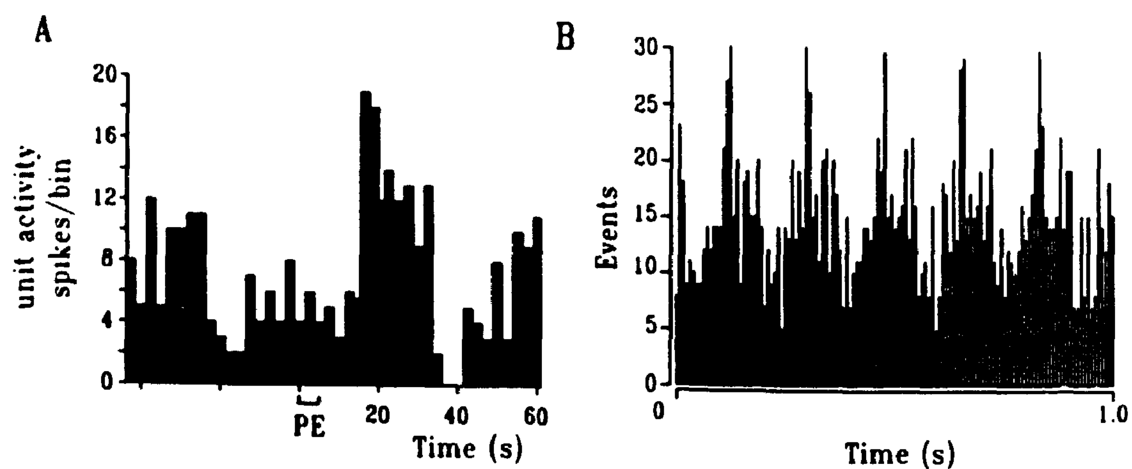

B
unit activity spikes/bin
6
5
4
3
2
1
0
20
40
60
80
100
120
GLY
Time (s)

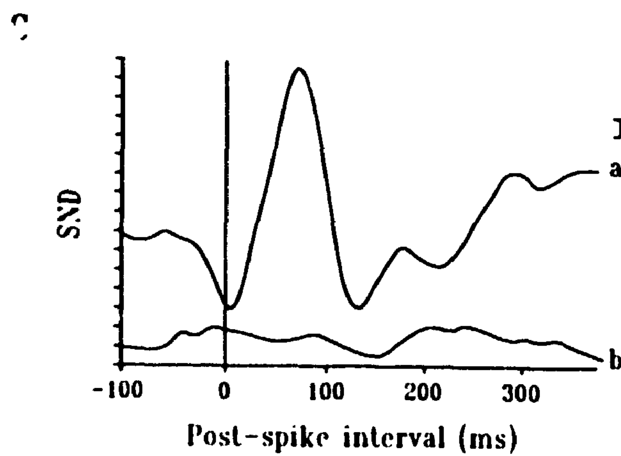
cardiovascular units. Discharge of 6 of these units was completely silenced by PRF blockade while discharge of 4 other units was reduced. The mean decrease in activity of these 10 cardiovascular units was 91 ±4% (from 2.7 ±0.3 spikes/s to 0.2 ±0.1 spikes/s). Activity of these units was inhibited within 3-15s after glycine injection and returned to control discharge rate within 20-115s. The duration of inhibition of unit activity (mean 59 ±9s) was not different from the duration of inhibition of renal nerve activity (mean 55 ±5s). Fig. 24B shows the effect of PRF blockade on the spontaneous activity of a cardiovascular unit located in the RVLM. The spontaneous discharge of 5 cardiovascular units was not affected by injection of glycine into the PRF.

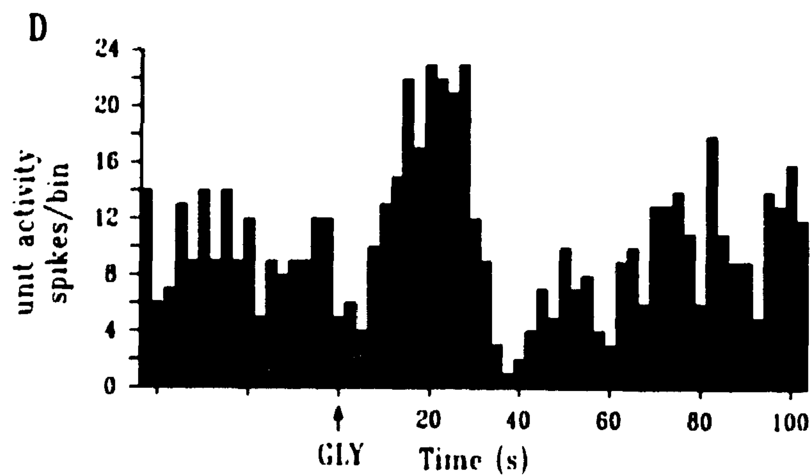
Activity of 3 additional units (firing rate 2.5-4.0 spikes/s) in the region of the RVLM was clearly synchronized to the cardiac cycle, but the discharge of these units was increased by 185-343% after baroreceptor activation elicited by an increase in arterial pressure. Two of these units also had activity correlated with renal nerve discharge. Unilateral injection of glycine into the PRF increased the spontaneous activity of 2 of these units by 139% and 125% and had no effect on the activity of 1 unit. Fig. 25 shows the responses to PRF blockade of 1 unit that was excited by baroreceptor activation and correlated with the cardiac cycle and renal nerve discharge.

In addition to the cardiovascular units, spontaneous activity was recorded from 6 other units in the region of the RVLM that did not meet the criteria for cardiovascular neurons. The firing rate of these neurons ranged from 3.4 to 14.0 spikes/s with a mean firing rate of 8.9 ±1.6 spikes/s. Four of these 6 units were likely associated with

Figure 25. Identification and response to blockade of the PRF of a unit in the region of the RVLM. A. spontaneous unit activity was increased by baroreceptor activation with phenylephrine injection (PE; bin width 2.5ms). B. post R-wave histogram of unit activity shows a cardiac rhythm (928 sweeps). C. spike-triggered (trace a) and "dummy" (trace b) averages of renal nerve activity (981 sweeps; bin width 1ms; vertical calibration 20μV). D. rate-meter histogram of spontaneous unit activity (bin width 2.5ms). Time is indicated on the x-axis. The ongoing activity of this cardiovascular unit was increased by glycine injection into the PRF (arrow).


A
unit activity
spikes/bin
PE
Time (s)
B
Events
Time (s)


SND
a
b
Post-spike interval (ms)


D
unit activity
spikes/bin
GLY
Time (s)

central networks controlling respiration since the firing of these neurons was synchronized to lung inflation and the recording sites were located in the region of the ventral respiratory group (Feldman & Ellenberger, 1988). Baroreceptor activation had no effect on the activity of these 4 units and their discharge was not synchronized with the cardiac cycle (Brown & Guyenet, 1985). The activity of these "respiratory" units was correlated with renal nerve discharge. Spontaneous activity of 2 other units was not affected by baroreceptor activation and was not correlated with the cardiac cycle or with renal nerve activity. Although glycine injection into the PRF caused decreases in arterial pressure, heart rate and renal nerve activity, these injections had no effect on the spontaneous activity of any of the 6 non-cardiovascular units. The lack of response of 1 non-cardiovascular unit to PRF blockade is shown in Fig. 26.

The locations of the microinjection sites in the PRF and the recording sites of RVLM cardiovascular and non-cardiovascular units are plotted on modified drawings (Paxinos & Watson, 1986) of transverse sections of the rat brainstem in Fig. 27.

Figure 26. Identification and response to blockade of the PRF of a non-cardiovascular unit located in the region of the RVLM. Format as in **Figure 25**. A. bin width 2.5ms. B. 1746 sweeps. C. 751 sweeps; bin width 1ms; vertical calibration 10 μV. D. bin width 2.5ms. The spontaneous activity of this unit was not affected by baroreceptor activation and was not correlated with the cardiac cycle or with renal nerve discharge. Injection of glycine (arrow) into the PRF had no effect on the ongoing activity of this unit.

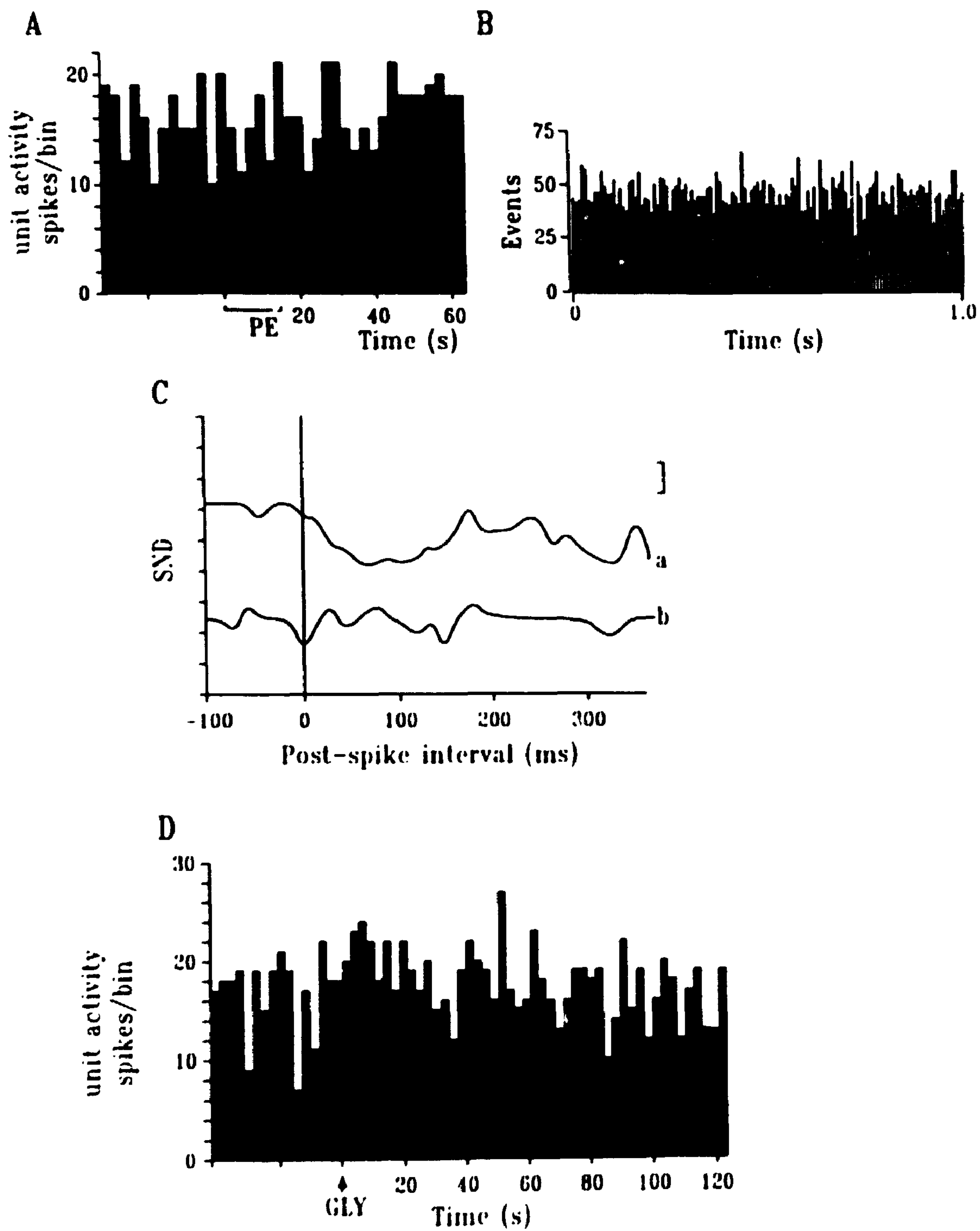
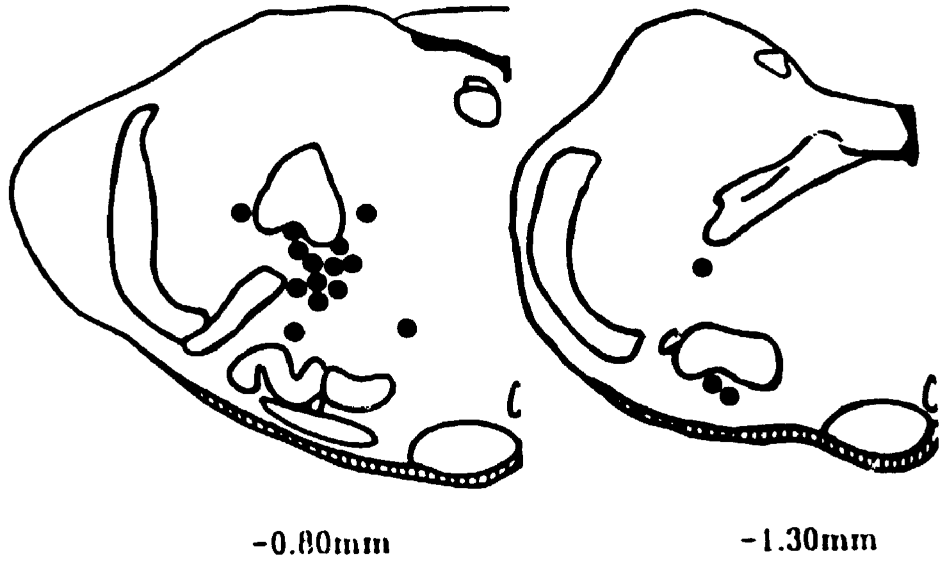
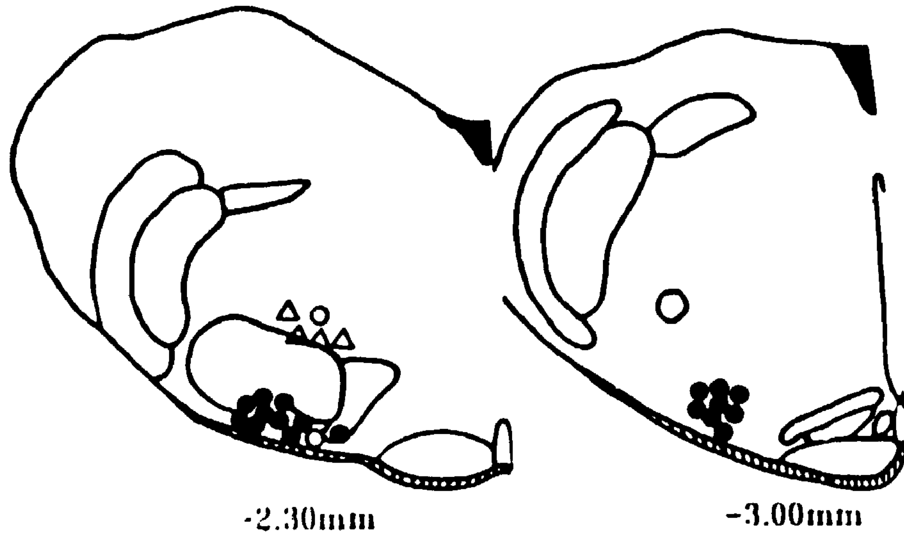

A
20
10
0
unit activity
spikes/bin
PE
20
40
60
Time (s)
B
75
50
25
0
Events
0
1.0
Time (s)
C
SND
a
b
-100
0
100
200
300
Post-spike interval (ms)
D
30
20
10
0
unit activity
spikes/bin
GLY
20
40
60
80
100
120
Time (s)

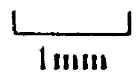
Figure 27. Drawings of transverse sections of rat pons (0.80 and 1.30 mm caudal to the interaural line) and medulla (2.30 and 3.00 mm caudal to the interaural line) showing locations of sites of injection and recording. A. Filled circles indicate sites in the pontine reticular formation in which glycine injections caused cardiovascular and sympathetic responses. B. Filled circles indicate sites of recording from cardiovascular units in the region of the RVLM; open circles indicate recording sites of non-cardiovascular units; open triangles indicate recording from non-cardiovascular units that displayed respiratory-related activity.

A. PRF



B. RVLM





5.4 Discussion

The most important finding of this study is that inhibiting the ongoing activity of pontine reticular neurons (PRF) eliminates or decreases the ongoing activity of cardiovascular neurons located in the rostral ventrolateral medulla (RVLM). These RVLM neurons are likely to be involved in vasomotor control since their spontaneous activity is silenced by activation of baroreceptors and is synchronized with the cardiac cycle (Brown & Guyenet, 1985; Agarwal *et al.*, 1990). These neurons are located within a region just caudal to the facial nucleus, bounded dorsally by the nucleus ambiguus and medially by the inferior olive. Previous studies have shown that some neurons in this region exhibiting these characteristics also have axonal projections to the intermediolateral cell column (IML) of the spinal cord (Brown & Guyenet, 1985; Morrison *et al.*, 1988). Although the cardiovascular neurons identified in the present study may have had axons that project directly to the IML or they may also have been local sympathoexcitatory interneurons. Of the 15 cardiovascular units in the RVLM, 7 showed correlation with renal nerve activity and 8 did not. The absence of correlation between the activity of the 8 units and renal nerve activity does not exclude a role for these neurons in vasomotor control. For example, other brainstem neurons known to contribute to central control of the sympathetic nervous system, such as raphe serotonergic neurons or some neurons in the A5 region, do not display sympathetic-related discharge (McCall & Clement, 1989; Huangfu *et al.*, 1991). In addition, a strong correlation of unit activity and sympathetic nerve activity may not have been observed because the predominant frequency component in sympathetic discharge was

not in the 2-6 Hz range. In 7 cases in which cardiovascular units did not display sympathetic-related discharge, power density spectral analysis revealed weak or absent 2-6 Hz rhythmicity in the discharge of renal nerves. Finally, since ventrolateral medullary neurons may be differentially correlated to different postganglionic nerves (Barman *et al.*, 1984), the discharge of these 8 units may have been correlated with activity of sympathetic nerves other than renal nerves. Correlation of unit activity with renal nerve discharge was not related to the response of that unit to PRF blockade. Only six of the 10 cardiovascular units inhibited by blockade of the PRF were also correlated with renal nerve discharge.

PRF blockade failed to change the ongoing activity of 5 cardiovascular neurons. One possible explanation is that these cardiovascular units receive excitatory drive from PRF neurons that were not inhibited by the glycine injection. Alternatively, not all cardiovascular units in the RVLM may receive synaptic input from neurons in the PRF.

Inhibition of RVLM unit activity could have been caused by the diffusion of glycine from the tip of the microinjection electrode in the PRF to the site of recording in the RVLM. However, this possibility is unlikely since neurons in the RVLM with activity that was inhibited by PRF blockade were located at a distance further from the site of microinjection than some neurons with discharge that was unaffected by PRF blockade. Furthermore, glycine injection into the PRF had no effect on the spontaneous discharge of 6 neurons that failed the criteria for cardiovascular neurons. Therefore, PRF blockade appears to have selectively inhibited some RVLM

cardiovascular neurons and not nearby units with other functions. For example, glycine injection into the PRF did not affect the activity of neurons that may be related to central respiratory systems even though neurons involved in central respiratory drive have been located in the pons (Feldman & Ellenberger, 1988). Finally, inhibition of RVLM cardiovascular unit activity was not an artifact of a dramatic fall in arterial pressure since neuronal firing could be silenced by PRF blockade when arterial pressure was not changed by glycine injection into the PRF (unpublished observation).

Although previous studies have shown that glycine inhibits the activity of some brainstem neurons for at least 40 min (Hayes & Weaver, 1990), blockade of the PRF caused only brief inhibition of RVLM cardiovascular units (<120s). Following loss of excitatory drive from the PRF, some RVLM neurons may resume firing independently due to their intrinsic pacemaker properties (Sun *et al.*, 1988a; 1988b) or RVLM activity may be generated by other excitatory synaptic inputs (Barman & Gebber, 1987; Gebber & Barman, 1988). The rapid recovery of RVLM neuronal activity is likely responsible for the recovery of arterial pressure and sympathetic nerve activity and may explain the transient nature of cardiovascular and sympathetic responses to PRF blockade. This hypothesis is supported by our finding that PRF blockade caused cardiovascular and sympathetic responses of the same duration as inhibition of RVLM unit activity.

Anatomical studies have identified some axonal projections from pontine reticular neurons to the ventrolateral medulla (Andrezik, Chan-Palay & Palay, 1981; Ruggiero

et al., 1989). However, the present results do not demonstrate a direct connection between the PRF and sympathoexcitatory neurons in the RVLM. The effects of glycine injection into the pons may be mediated by other neural structures or by local interneurons. For example, the 3 neurons that showed spontaneous activity correlated with the cardiac cycle and that were excited by activation of baroreceptors might represent inhibitory interneurons in the region of the RVLM. Since PRF blockade increased the spontaneous activity of 2 of these units, the inhibitory effect on sympathoexcitatory neurons could be mediated by these putative interneurons.

Although our results demonstrate that the ongoing activity of neurons in the PRF provides tonic excitation to RVLM cardiovascular neurons, we have not determined whether vasomotor activity originates from neurons in the PRF. While PRF neurons may indeed be the source of this drive, it is also possible that they are relay neurons from drive generated elsewhere in the neuraxis such as the forebrain (Huang *et al.*, 1987; 1988), or other brainstem areas (Gebber & Barman, 1988; Yardley *et al.*, 1989b; Korkola & Weaver, 1992). Barman and Gebber (1987; Gebber and Barman, 1988) have shown in the cat that a group of sympathoexcitatory neurons in the lateral tegmental field (LTF) acts as an important source of excitatory inputs to RVLM neurons innervating the IML. While the PRF does not appear to be a rostral extension of the LTF, PRF neurons may contribute to or receive inputs from a brainstem reticular network that is collectively responsible for generating sympathetic activity.

To our knowledge this study has provided the first direct evidence that the ongoing activity of cardiovascular neurons in the RVLM is supported by an excitatory

drive. Furthermore, ongoing activity of neurons in the pontine reticular formation is one source of this tonic excitation to some RVLM cardiovascular units. In the absence of excitatory influences from the PRF, RVLM neurons are still capable of tonic discharge due to their own intrinsic properties or to other synaptic inputs.

Chapter 6: Axonal projections of pontine reticular neurons involved in tonic cardiovascular control identified with the anterograde tracer *Phaseolus vulgaris leucoagglutinin* in rats.

Neuroscience, 1993. Submitted.

6.1 Introduction

The activity of neurons located in the pontine reticular formation (PRF) makes a significant contribution to tonic excitation of sympathetic nerves supporting arterial blood pressure and heart rate. In rats, inhibition of activity of PRF neurons by microinjections of the inhibitory amino acid, glycine or the $GABA_A$ agonist, muscimol produces large decreases in arterial pressure, heart rate and sympathetic nerve activity (Hayes & Weaver, 1992; Krassioukov & Weaver, 1992; Krassioukov, Gelb & Weaver, 1992). However, the neural pathways mediating these responses from the PRF are unknown.

Pontine reticular neurons may project to the rostral ventrolateral medulla (RVLM), a region that is critical for tonic cardiovascular control (review: Calaresu & Yardley, 1988; Barman, 1990; Guyenet, 1990; McCall, 1990; Chalmers & Pilowsky, 1991) and that contains neurons whose axons terminate in the intermediolateral cell column (IML) of the spinal cord (Ruggiero *et al.*, 1989). Evidence from recent electrophysiological studies indicates that a projection from the PRF to the RVLM may exist. Synaptic blockade within the RVLM with cobalt chloride or kynurenate eliminates the cardiovascular and sympathetic responses caused by glycine injection

into the PRF (Krassioukov & Weaver, 1992) suggesting that sympathetic influences from the PRF are relayed by RVLM neurons. Furthermore, since glycine injections into the PRF inhibit the spontaneous firing of some RVLM cardiovascular neurons (Hayes, Calaresu & Weaver, 1993), cardiovascular and sympathetic responses to PRF blockade may be due to the loss of tonic excitatory drive to RVLM sympathoexcitatory neurons.

Neurons in the PRF could influence vasomotor tone by projections to other brainstem areas involved in generation of sympathetic nerve activity such as the dorsal medullary reticular formation (Barman and Gebber, 1987; Yardley *et al.*, 1989b; Korkola & Weaver, 1992) and may also project directly to sympathetic preganglionic neurons in the IML of the spinal cord. The present study was done to determine the axonal projections of neurons in the PRF that may be involved in tonic cardiovascular and sympathetic regulation.

6.2 Methods

Surgical procedures.

Male Wistar rats (275-325g; Charles River, Canada) were anaesthetized initially with sodium pentobarbital (40 mg/kg, i.p.; M.T.C. Pharmaceutical, Canada) and the right femoral artery and vein were cannulated for monitoring arterial blood pressure and for delivery of anaesthetic respectively. An intravenous infusion (0.4-1.0 ml/hr) of the steroid anaesthetic Saffan (alphaxalone 9 mg/ml and alphadolone 3 mg/ml, Glaxo Canada Ltd., Canada) was begun as effects of the pentobarbital disappeared and adjustments in the rate of infusion were made as needed to maintain a constant plane of anaesthesia. The animal's plane of anaesthesia was assessed by examining palpebral and withdrawal reflexes. Body temperature was maintained at 37°C with a heating pad. The rats were placed in a Kopf stereotaxic frame equipped with blunted ear bars and a small hole was drilled in the parietal bone.

Microinjection procedures.

Glass micropipettes were pulled to a tip size of about 30 μm and filled with a solution of the inhibitory amino acid glycine (1.0M; pH=7.4; BDH Chemicals, Canada) dissolved in distilled water. The tips of the pipettes were positioned within the pontine reticular formation (PRF) according to a stereotaxic atlas (Paxinos & Watson, 1986) and glycine (22-43 nl) was microinjected unilaterally into the PRF using a picospritzer (General Valve Corporation, USA). Injection volumes were determined by the displacement of the meniscus at the air-liquid interface in the pipette observed through a microscope containing an ocular micrometer. Arterial

pressure and heart rate were recorded on a polygraph (Grass Instrument Company, USA). In 6 animals, a site within the PRF in which glycine caused decreases in arterial pressure and heart rate was identified. In 2 control animals, a site was located rostral to this region in which glycine failed to produce changes in arterial pressure or heart rate.

Anterograde tracing procedures.

Once a PRF or control site was identified, the pipette was retracted, emptied and filled with the anterograde tracer, *Phaseolus vulgaris leucoagglutinin* (PHA-L; 2.5% in 10 mM sodium phosphate; pH=8.0; Vector-Dimension Laboratories; Gerfen & Sawchenko, 1984). The pipette was repositioned and PHA-L was iontophoresed into the original site of microinjection (7 μA, 5s on-off cycle for 30 min). The pipette was retracted, the exposed surface of the brain was covered with sterile Gelfoam (Upjohn, Canada) and the incision was sutured. The arterial and venous cannulae were removed and this incision was also sutured. The animal was given penicillin (60 000 units, i.m., Veticare, USA).

A survival time of 7 days was allowed for 7 rats including all control animals. One rat survived for 14 days to determine the effect of increasing survival time on the pattern and quantity of PHA-L labelling. The rats were then deeply anaesthetized with urethane (1.4 g/kg, i.p; Aldrich Chemical Company, USA) and perfused transcardially with 250 ml of 10mM phosphate buffered saline, followed by 250 ml of a filtered solution of 4% paraformaldehyde in 0.1M sodium acetate (pH=6.5), followed by 250 ml of a filtered solution of 4% paraformaldehyde in 0.1M sodium borate (pH=10.0).

All solutions were at 4°C and all were prepared immediately before the perfusion. Spinal cord segments were identified by their dorsal roots and marked with a piece of suture and then the brain and spinal cord were removed and placed in a solution of 4% paraformaldehyde in 0.1M sodium borate for 24 hrs post-fix followed by 24 hrs in 30% sucrose solution. The first and eighth thoracic (T_1, T_8) and first lumbar (L_1) spinal cord segments were sectioned at 20 μm thickness and the brainstems were sectioned at 40 μm thickness.

Immunocytochemistry

Sections were rinsed three times in Tris buffered saline (0.05 M TBS; 5 min each) and incubated in a blocking solution (2% bovine serum albumin and 10% normal swine serum in a solution of TBS containing 0.3% Triton-X) for 1 hr at room temperature to eliminate nonspecific antibody labelling. Sections were then incubated in a primary antibody solution containing 0.05 M TBS with 0.3% Triton-X with gentle agitation for 48 hours at 4°C. The primary antiserum, rabbit anti-PHA-L (Dakopatts, Vector-Dimension Laboratories) was used at a dilution of 1:500. Sections were then rinsed three times in 0.05 M TBS and were placed in swine anti-rabbit antiserum (1:50 dilution; Dakopatts) for 1 hr at 4°C. Following three rinses in 0.05 M TBS, sections were incubated for 1.5 hrs in a solution containing a soluble peroxidase-antiperoxidase complex (Dakopatts) made in rabbit. Following this incubation, sections were again rinsed three times in 0.05 M TBS and incubated in a solution of diaminobenzidine tetrahydrochloride (0.05%; Sigma) and H_2O_2 (0.003%) until a visible reaction product appeared (about 30 min). Sections were mounted on glass slides and viewed with a

Leitz microscope (Wild-Leitz, Canada) for PHA-L labelled cell bodies at the injection site and PHA-L labelled axons and terminals within the brainstem and spinal cord. Swellings that were observed along the course of labelled fibres were considered to be putative terminal boutons and to indicate termination fields of PHA-L-labelled neurons. Plots of PHA-L labelling were produced by outlining landmarks and plotting labelled cell bodies and fibres using a drawing tube at low power (63X). Labelling of cell bodies, fibres and terminal boutons was confirmed at a higher power (250X and 400X).

Statistical analysis

Arterial pressure and heart rate responses for individual PRF responses were determined using 95% confidence intervals. A one-way analysis of variance (ANOVA) with repeated measures was used to determine statistical changes in mean arterial pressure and heart rate after glycine injection into the PRF. Differences were considered significant when $p<0.05$ and variability was expressed as the pooled standard error derived from ANOVA or as the standard error of the mean (Snedecor & Cochran, 1980).

6.3 Results

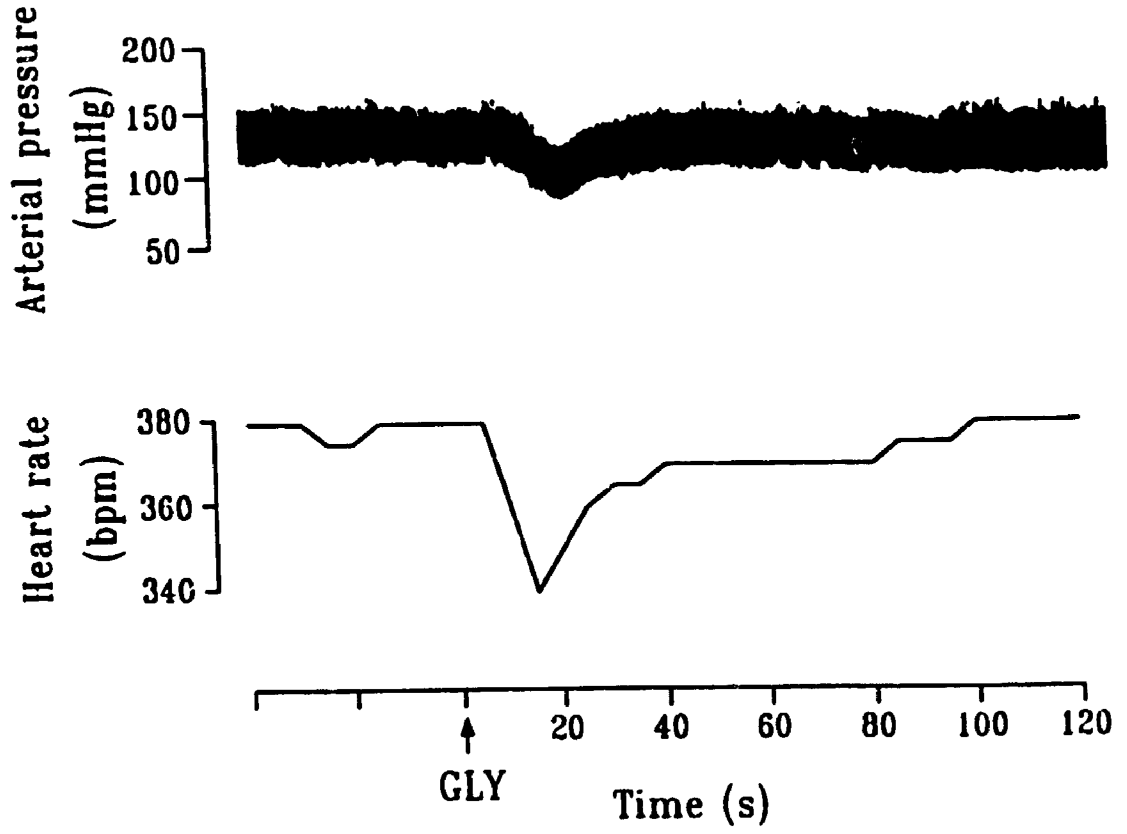
6.3.1 Anatomy of the pontine reticular formation (PRF).

The region of the PRF from which injections of glycine produce cardiovascular and sympathetic responses extends rostrally from the level of the caudal pole of the trapezoid body to the rostral pole of the motor nucleus of the trigeminal nerve. This region is bounded laterally by the motor nucleus of the trigeminal nerve, dorsally by the dorsal surface of the brainstem and ventrally by the superior olivary nucleus (Hayes & Weaver, 1992; present study). Although the PRF is comprised of several reticular nuclei, this functionally defined group of neurons is composed mainly of the pontine reticular nucleus (caudal part; PnC). The PnC contains mostly small and medium sized neurons with a few large cells interspersed among them (Andrezik & Beitz, 1985). The PnC can be distinguished from other reticular nuclei because within the cell body, the nucleus is located eccentrically and the Nissl substance is evenly distributed throughout the cytoplasm (Andrezik & Beitz, 1985). In addition, the PnC contains a collection of giant neurons (50-65 μm diameter) in the medial portion (Andrezik & Beitz, 1985). The PnC is coextensive with the gigantocellular reticular nucleus and some cardiovascular responses have been produced by glycine injections into the rostral portion of this nucleus.

6.3.2 Cardiovascular responses to glycine injection into the PRF.

An example of the arterial pressure and heart rate responses produced by a unilateral injection of glycine into the PRF is shown in Fig. 28. Unilateral injections (n=6) into the PRF produced brisk decreases in arterial pressure and heart rate that

Figure 28. Responses to a unilateral microinjection (35 nl) of glycine (1.0M) into the pontine reticular formation of one rat. Top panel: record of systemic arterial blood pressure; Bottom panel: heart rate. Time is indicated beneath these panels. Injection of glycine (arrow) produced decreases in arterial pressure and heart rate that returned to control values within 100s.



Arterial pressure
(mmHg)
200
150
100
50
Heart rate
(bpm)
380
360
340
20
40
60
80
100
120
GLY
Time (s)

Figure 10. Response of one rat to cervical spinal cord transection (C1X). A: systemic arterial blood pressure is illustrated in the top panel. Integrated activity from renal and splenic nerves (recorded simultaneously is illustrated in the second panel. Time is indicated beneath these panels. PE indicates start of phenylephrine infusion, which was continued throughout the experiment. Letters above each panel refer to time period in which neurograms in part B were taken. B: neurogram of renal (RNA;■) and splenic (SNA;Δ) sympathetic nerves before (a) and 60 s (b), 5 min (c), 15 min (d) and 30 min (e) after cord transection. Neurograms c and d were taken at the end of each sample period. Vertical calibration is 20 μV.

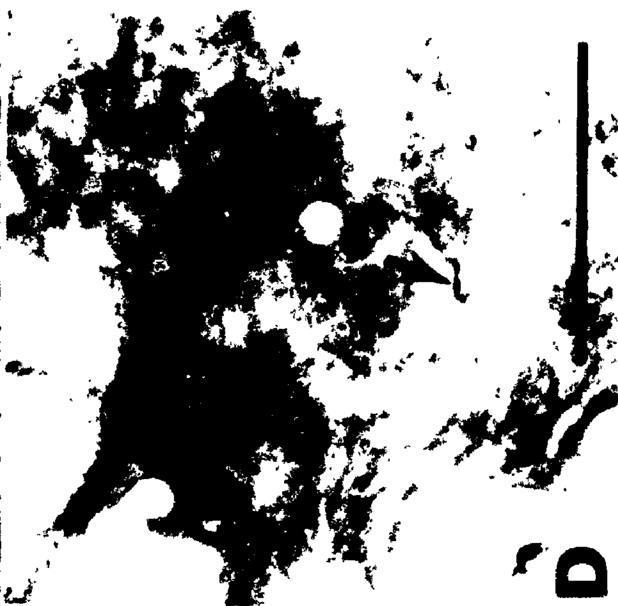
began within 5-10 seconds, reached a maximum decrease in 10-25s and returned to control values within 40-60s. Mean arterial pressure was decreased by 25 ±3 mmHg (from 99 to 74 mmHg; pooled S.E.=2.0) and heart rate was decreased by 24 ±4 bpm (from 359 to 335 bpm; pooled S.E.=2.3) following glycine injection. In 2 control animals glycine was injected into sites rostral to the region of the PRF. The mean control arterial pressure (96 ±2 mmHg) and mean control heart rate (360 ±1 bpm) of these animals were not different from those in which glycine was injected into the PRF. Glycine injection into control sites had no effect on arterial pressure or heart rate.

6.3.3 Anterograde labelling of *Phaseolus vulgaris leucoagglutinin* (PHA-L).

Injection sites. A dark reaction product could be seen within cell bodies that had incorporated PHA-L at the injection site (Fig. 29a). Large PHA-L injections labelled between 100 and 140 neurons within an area with a diameter of about 400μm while smaller injections labelled 25 to 70 neurons within an area with a diameter of about 200μm. The locations of PHA-L injection sites are shown in Fig. 30. Six injections of PHA-L of different sizes were made into sites from which glycine evoked cardiovascular responses. Five of these sites (cases 1, 2, 4, 8 and 9) were located within the pontine reticular nucleus (caudal part) and were used for the description of the efferent projections from PRF neurons involved in cardiovascular control. Three of these injections (cases 1, 8 and 9) also labelled some cells in the parvocellular reticular formation. One PHA-L injection (case 3) was located in the ventrolateral

Figure 29. Brightfield photomicrographs of A) a site of injection of PHA-L into the pontine reticular formation (case 1) and PHA-L labelled axons and terminals within B) medullary reticular formation, C) rostral ventrolateral medulla and D) intermediolateral cell column of the first thoracic spinal cord segment. Arrows show examples of terminal boutons along labelled fibres. Calibration bars are 100 μm. 7n (facial nerve).

A


B

C

D

Figure 30. Series of drawings of transverse sections of rat pons (0.70 mm rostral and 0.70 mm and 1.00 mm caudal to the interaural line) showing the locations and diameters of the PHA-L injections into the PRF and surrounding areas. Numbers refer to the different cases. Aq (aqueduct); CG (central gray); PnO (pontine reticular nucleus, oral part); RPO (rostral periolivary region); CGPn (central gray pons); PB (parabrachial nucleus); LC (locus coeruleus); Mo5 (motor trigeminal nucleus); Pr5 (principal sensory trigeminal nucleus); PnC (pontine reticular nucleus, caudal part); A5 (region of the A5 cell group); 7n (facial nerve); PnV (pontine reticular nucleus, ventral part); RMg (raphe magnus); PCRt (parvocellular reticular nucleus).

Aq
CG
PnO
6
5
RPO
1 mm
+0.70 mm

CGPn
PB
LC
Mo5
Pr5
2
4
PnC
A5
7n
RMg
PnV
−0.70 mm

LC
7n
PCRt
1
8
Pr5
9
PnC
3
A5
PnV
RMg
1 mm
−1.00 mm

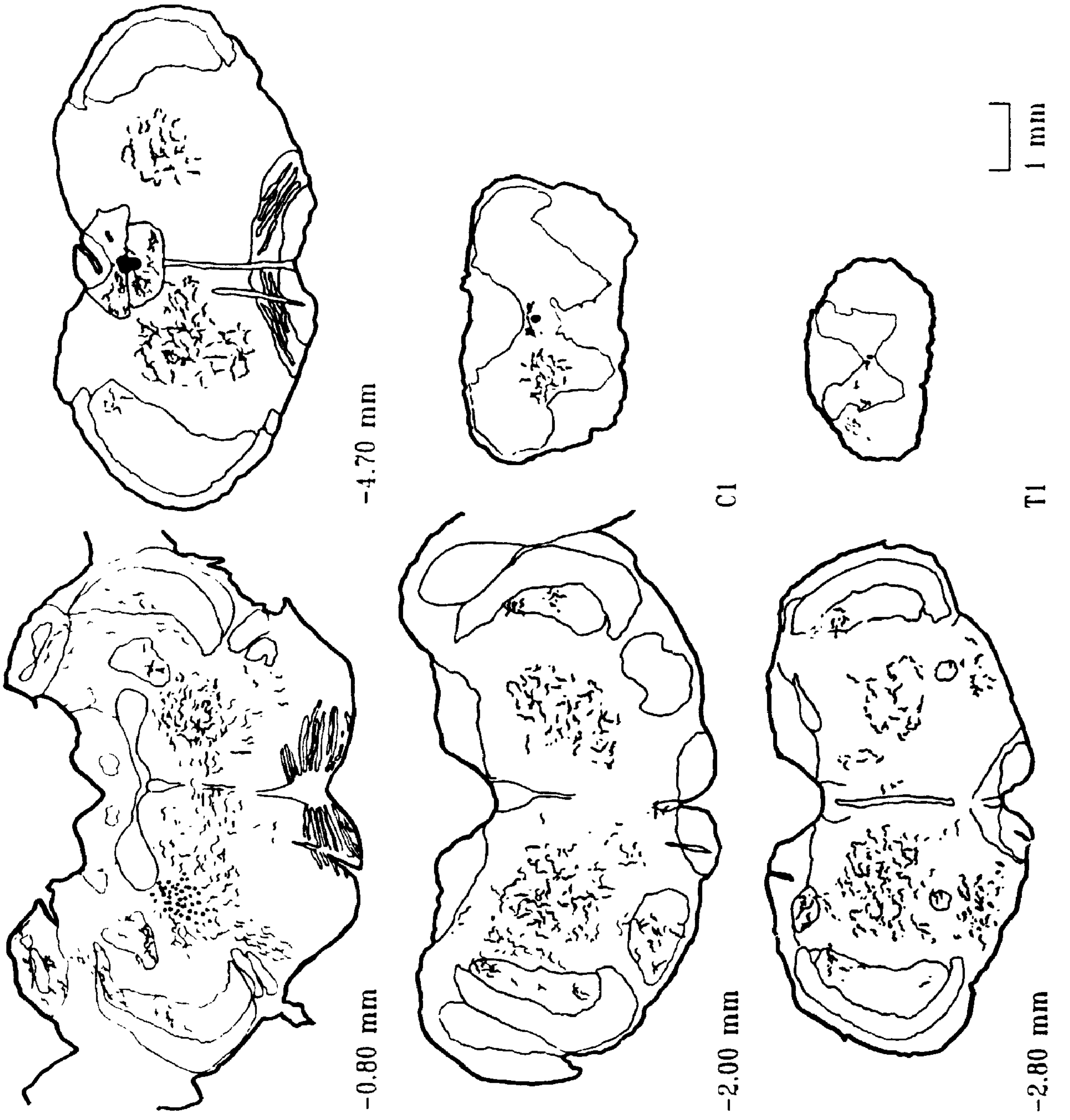
pons within the region of the A5 cell group and labelled projections from this region were compared to those caused by PHA-L injections located exclusively in the PRF. Two control PHA-L injections (cases 5 and 6) were made into sites from which glycine failed to evoke cardiovascular responses and these sites were located at the level of the aqueduct within the pontine reticular nucleus (oral part). These control injections were the same size and labelled the same number of neurons as injections made into sites within the pontine reticular nucleus (caudal part). The results of two experiments (cases 7 and 10) were not used as these PHA-L injections did not produce sufficient anterograde labelling for analysis.

6.3.4 Efferent pathways of pontine reticular neurons.

After injections of PHA-L into the PRF, PHA-L - positive fibres and terminal boutons could be identified in many nuclei of the pons and medulla as well as in the cervical and thoracic spinal cord. The sites of nuclei receiving projections from PRF neurons are illustrated with camera lucida drawings of a representative PHA-L injection (case 1) in Fig. 31. The brainstem and spinal cord sites of projection and the quantity of anterograde labelling within these areas were identical in animals with survival times of 7 and 14 days.

Nuclei in the pons. PHA-L injections into the PRF resulted in dense anterograde labelling of fibres throughout the ipsilateral pontine reticular formation extending caudal from the level of the aqueduct to the pontomedullary border. A considerable number of labelled axons exiting the injection site projected across the midline to the contralateral PRF where dense PHA-L labelling of axons and terminals were observed.

Figure 31. Series of camera lucida drawings of transverse sections of rats pons, medulla, cervical (C1) and first thoracic (T1) spinal cord illustrating the injection site and course of labelled fibres and the termination fields of a representative PHA-L injection into the pontine reticular formation (case 1). Numbers indicate distance from interaural zero. Refer to Figure 32 for labelling of specific neural structures.



−4.70 mm
C1
T1
1 mm
−0.80 mm
−2.00 mm
−2.80 mm

Anterograde labelling of axons and terminals within the ipsilateral parabrachial complex was located mainly within the central lateral and external lateral subnuclei but diffuse labelling of fibres and terminals was also observed within the medial parabrachial nucleus (Fig. 32a&b). Labelling within the contralateral parabrachial complex was confined to the medial subnucleus. PHA-L labelled axons and terminals were also located within the parvocellular reticular formation and in the motor trigeminal nucleus. After PHA-L injections that had incorporated cells in the parvocellular reticular formation (cases 1, 8 and 9), PHA-L - positive fibres were also observed within the principal sensory trigeminal nucleus. Although PHA-L labelling was observed on sides both ipsilateral and contralateral to the injections site, contralateral labelling was much less dense.

The distribution of labelled axons and terminals within the pons following injections of PHA-L into the A5 region or into sites rostral to the PRF was very different from that produced by injections into sites within the PRF. The sites of nuclei receiving projections from neurons located within the A5 region (case 3) are shown in Fig. 33. After one injection of PHA-L into the region of the A5 cell group, anterograde labelling of fibres and terminals was observed within the medial subnucleus of the ipsilateral parabrachial complex but not within the reticular formation or any other pontine nuclei.

Although control injections rostral to the PRF produced PHA-L labelling of fibres and terminals throughout the pontine reticular formation and sparse labelling of the parabrachial complex, dense labelling of fibres was also located within the central gray

Figure 32. Darkfield and brightfield photomicrographs of PHA-L labelled axons and terminals within the parabrachial nucleus (A and B) and nucleus tractus solitaris (C and D) following a PHA-L injection into the pontine reticular formation (case 1). The darkfield photomicrographs are taken at lower magnification. Arrows in the darkfield photomicrographs (A and C) identify the same PHA-L labelled fibres as indicated by the arrows in the brightfield photomicrographs (B and D). Calibration bars are 100 μm. vsc (ventral spinocerebellar tract); scp (superior cerebellar peduncle); 4v (4th ventricle); sol (solitary tract).

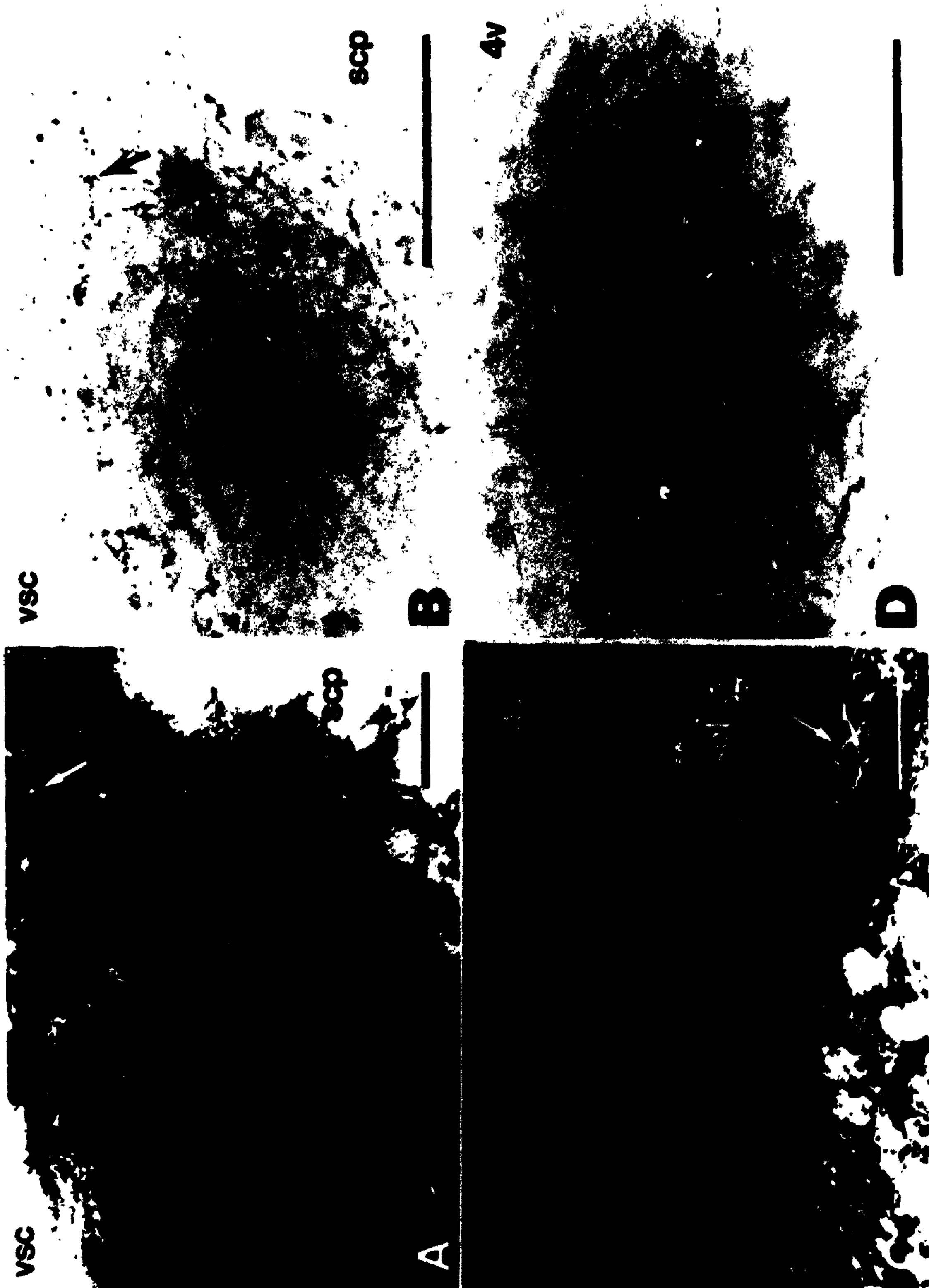
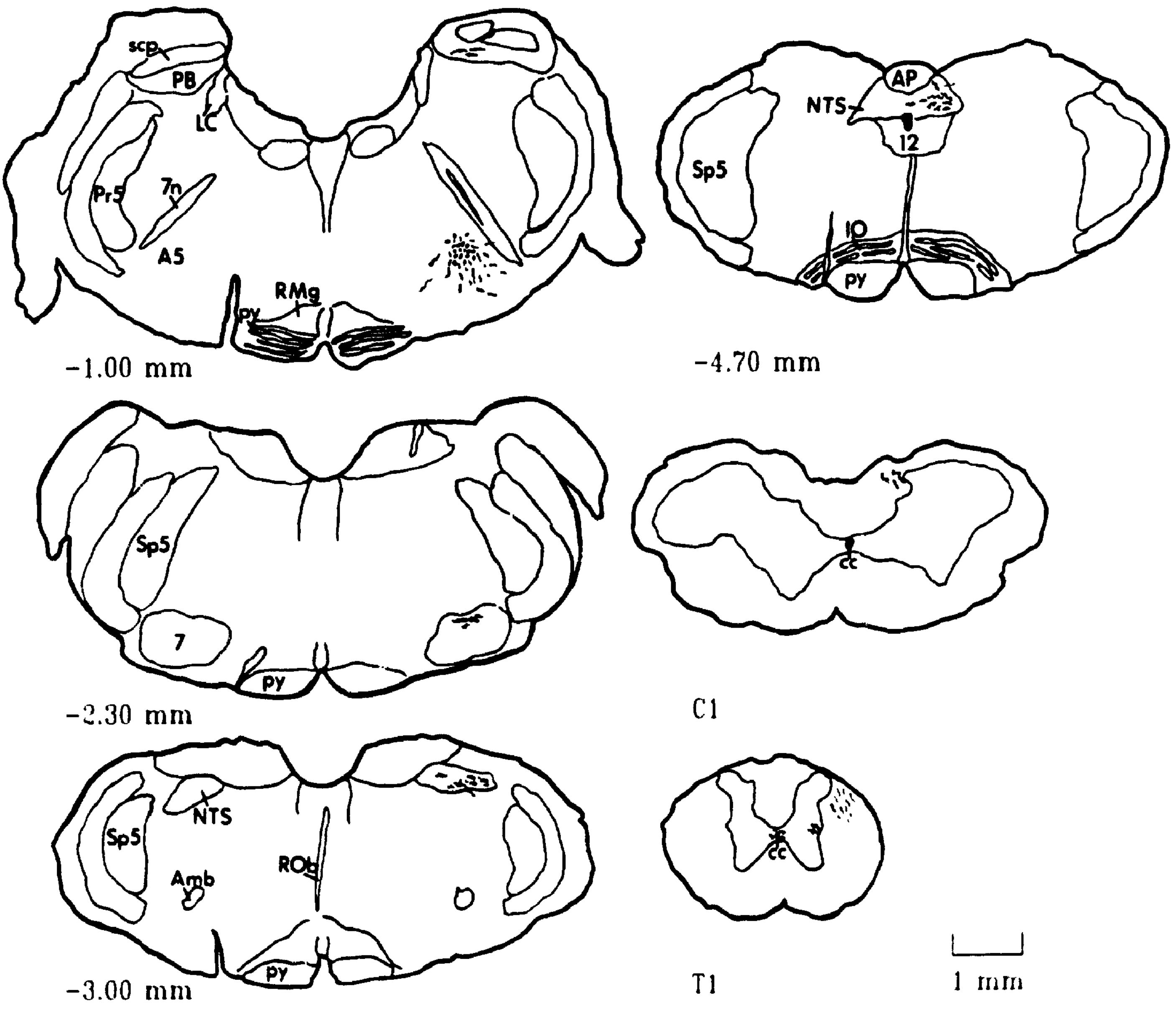

vsc
scp
vsc
scp
4v
A
B
D

Figure 33. Series of camera lucida drawings of transverse sections of rat pons, medulla, cervical spinal cord (C1) and first thoracic (T1) spinal cord illustrating the injection site and course of labelled fibres and termination fields of a PHA-L injection into the region of the A5 cell group (case 3). Numbers indicate distance from interaural zero. PB (parabrachial nucleus); scp (superior cerebellar peduncle); LC (locus coeruleus); Pr5 (principal sensory trigeminal nucleus); 7n (facial nerve); A5 (region of the A5 cell group); RMg (raphe magnus); py (pyramidal tract); Sp5 (spinal trigeminal nucleus); 7 (facial nucleus); NTS (nucleus tractus solitaris); Amb (nucleus ambiguus); ROb (raphe obscurus); AP (area postrema); 12 (hypoglossal nucleus); IO (inferior olive); cc (central canal).


scp
PB
LC
Pr5
7n
A5
RMg
py
-1.00 mm
AP
NTS
12
Sp5
IO
py
-4.70 mm
Sp5
7
py
-2.30 mm
cc
C1
NTS
Sp5
Amb
ROb
py
-3.00 mm
cc
T1
1 mm

nucleus of the pons, raphe magnus and the pontine reticular nucleus (ventral; PnV). PHA-L labelled fibres were also observed within the ipsilateral and contralateral locus coeruleus.

Nuclei in the medulla. PHA-L injections into the PRF resulted in dense anterograde labelling of axons and terminals throughout the medullary reticular formation from the pontomedullary border to the cervical spinal cord (Fig. 29b). PHA-L positive labelling was most dense on the side ipsilateral to the injection site, although labelled fibres were also found within the contralateral reticular formation. PHA-L injections into the PRF yielded a substantial number of PHA-L labelled fibres and boutons throughout the region of the rostral ventrolateral medulla (RVLM; Fig. 29c). Axonal projections from the PRF were directed predominantly to the ipsilateral RVLM extending beneath the nucleus ambiguus to the ventral surface of the medulla but a few labelled axons and terminals were found in the contralateral RVLM. PHA-L labelled axons and terminals were also observed within the ipsilateral and contralateral hypoglossal nucleus and within ventral and medial portions of the nucleus tractus solitaris (NTS) on the ipsilateral side only (Fig. 32c&d). After PHA-L injections that had labelled cells within the parvocellular reticular formation (cases 1, 8 and 9), labelled fibres were found within the ipsilateral and contralateral caudal spinal trigeminal nucleus and facial nucleus.

Injections of PHA-L into the A5 region or into control sites produced a pattern of anterograde labelling very different from that caused by PHA-L injections into sites within the PRF. One PHA-L injection into the A5 region caused labelling of axons

and terminals within the ipsilateral NTS but failed to label fibres within the medullary reticular formation including the ventrolateral medulla. A few labelled fibres were located within the ipsilateral facial nucleus.

After PHA-L injections into sites rostral to the PRF, dense labelling of fibres and terminals was observed within the gigantocellular reticular nucleus (alpha; GiA), which is a continuation of the PnV in the pons and in raphe magnus. Less dense labelling of the dorsal medullary reticular formation, raphe obscurus and raphe pallidus was also observed. PHA-L positive fibres and terminals were absent in the region of the ventrolateral medulla.

Spinal cord. After injections of PHA-L into the PRF, dense anterograde labelling of fibres and terminals was observed within lamina VII of the ipsilateral cervical spinal cord and fewer PHA-L positive axons and terminals were also found in laminae V, VIII and X. Few labelled fibres were found in the contralateral cervical spinal cord. PHA-L injections into the PRF resulted in a few anterogradely labelled fibres in the thoracic spinal cord. The majority of PHA-L labelled fibres in the spinal gray matter were located in the ipsilateral intermediolateral cell column (IML) but some labelled fibres were also found in the central autonomic nucleus, in lamina V and in other regions of lamina VII. Terminal boutons were observed along all fibres in the spinal gray matter indicating that these fibres may make synaptic connections in these areas. An example of an anterogradely labelled fibre within the IML is shown in Fig. 29d. Labelled axons without swellings were only found in the ipsilateral dorsolateral funiculus. PHA-L labelling on the side contralateral to the injection site was never

observed. Spinal cord labelling was never observed in lumbar spinal cord segments.

One injection of PHA-L into the A5 region labelled fibres within the dorsolateral funiculus but fibres and terminals within the gray matter of the cervical spinal cord were not observed. This injection also resulted in PHA-L labelled fibres within the IML and central autonomic nucleus.

PHA-L injections into sites rostral to the PRF caused dense labelling of fibres and terminals in laminae IV and VII and sparse labelling in laminae V, VIII and X of the ipsilateral cervical cord. A few PHA-L positive fibres were observed in the contralateral cervical cord. These injections also caused labelling within laminae IV, V and VII and within the dorsolateral funiculus of both ipsilateral and contralateral thoracic spinal cord. These injections did not produce PHA-L positive fibres within the IML or central autonomic nucleus.

6.4 Discussion

The major efferent projections of pontine reticular (PRF) neurons involved in tonic cardiovascular control were identified in the present study. Anterograde transport of *Phaseolus vulgaris leucoagglutinin* (PHA-L) from PRF neurons labelled axons and terminals in several brainstem areas that have been shown to be involved in regulation of the cardiovascular system including central lateral, external lateral and medial subnuclei of the parabrachial complex (Mraovitch *et al.*, 1982; Fulwiler & Saper, 1984; Herbert, Moga & Saper, 1990; Miura & Takayama, 1991), medullary reticular formation (Barman and Gebber, 1987; Yardley *et al.*, 1989; Korkola & Weaver, 1992), nucleus tractus solitaris (NTS: Spyer, 1981) and rostral ventrolateral medulla (RVLM; review: Calaresu & Yardley, 1988; Barman, 1990; Guyenet, 1990; McCall, 1990; Chalmers & Pilowsky, 1991). A few projections from PRF neurons also terminated within regions of the spinal cord associated with autonomic functions including laminae VII and X of the cervical spinal cord and within the intermediolateral cell column (IML), central autonomic nucleus and laminae V and VII of thoracic spinal cord segments. The dorsal medullary reticular formation and the RVLM have been implicated in generation of ongoing sympathetic nerve activity (Barman and Gebber, 1987; Sun *et al.*, 1988a; 1988b; Korkola & Weaver, 1992). Therefore, PRF neurons could influence tonic vasomotor outflow by synaptic connections in either of these brainstem areas as well as by direct projections to the sympathetic preganglionic neurons in the spinal cord.

Pontine reticular neurons involved in tonic cardiovascular regulation appear to

have axonal projections distinct from those of nearby cell groups. Control injections of PHA-L into sites from which glycine did not elicit cardiovascular responses resulted in anterograde labelling of fibres and terminals within the brainstem and spinal cord but not within the ventrolateral medulla or within the IML. This distinct pattern of anterograde labelling was not due to differences in the size of PHA-L injection, in the number of neurons labelled at the injection site or in the survival time of these control animals. Furthermore, neurons located within the rostral parvocellular reticular formation, located lateral to the PRF have few projections that terminate within the ventrolateral medulla (Ter Horst, Copray, Liem & Van Willigen, 1991). This cell group is primarily involved in orofacial motor control (Minkels, Jüch, Ter Horst & Van Willigen, 1991; Ter Horst *et al.*, 1991). Finally, the A5 cell group, a collection of noradrenergic cells in the ventrolateral portion of the PRF, has known cardiovascular functions and has major projections to the NTS and to the IML of the spinal cord (Byrum *et al.*, 1984; Stanek *et al.*, 1984; Byrum and Guyenet, 1987; Huangfu *et al.*, 1992). In contrast to PRF neurons, A5 neurons provide only sparse innervation of the RVLM (Loewy, *et al.*, 1986; Byrum and Guyenet, 1987). In the present study the injection of PHA-L into the region of the A5 cell group may have labelled neurons other than A5 cells since noradrenergic neurons are dispersed among other reticular neurons in this region. However, this injection site was located in an area shown to contain cell bodies immunoreactive for tyrosine hydroxylase (Byrum & Guyenet, 1987). In addition, some A5 neurons within this injection site probably incorporated PHA-L because anterograde labelling within the brainstem and spinal

cord was similar to that observed in previous studies of projections of A5 cells. Furthermore, this pattern of labelling was distinct from that produced by PHA-L injections into nearby reticular sites. This evidence suggests that the PRF contains a group of neurons that can be distinguished from other pontine neurons by their role in cardiovascular control and by their efferent connections.

Axonal projections of reticular neurons in the region of the PRF have been demonstrated as part of other anatomical investigations. Previous studies have shown that neurons in this region project to the parabrachial nuclear complex, Kolliker-Fuse nucleus, RVLM and spinal trigeminal nuclei (Andrezik *et al.*, 1981; Ruggiero *et al.*, 1989; Ter Horst *et al.*, 1991) but do not have axons that terminate in the NTS (Ter Horst *et al.*, 1991). Spinal cord projections from PRF neurons have been located in the IML (Blessing *et al.*, 1981b) and in both dorsolateral and ventrolateral funiculi (Carlton, Chung, Leonard & Willis, 1985). However, axons from PRF neurons travelling in the ventral and ventrolateral funiculi are probably involved in mediating locomotory responses originating in the mesencephalic motor area (Noga, Kriellaars & Jordan, 1991).

Innervation from either the left or right PRF was directed predominantly to ipsilateral brainstem regions. This anatomical evidence supports electrophysiological studies that suggest that sympathetic influences descending from the PRF depend primarily on synaptic connections within the ipsilateral ventrolateral medulla. Unilateral microinjection of the synaptic blocking agent cobalt chloride into the RVLM eliminated sympathetic responses to injection of glycine into the ipsilateral

PRF (Krassioukov & Weaver, 1992). Conversely, unilateral synaptic blockade within the RVLM had no effect on responses to glycine injection into the contralateral PRF (Krassioukov & Weaver, 1992). Furthermore, since PRF responses are completely abolished by synaptic blockade within the RVLM, the sparse spinal projection from pontine reticular neurons probably plays little role in mediating sympathetic influences from the PRF.

Unilateral PHA-L injections into the PRF resulted in dense anterograde labelling of axons and axon terminals in the contralateral PRF and a considerable number of axons exiting the injection site were observed crossing the midline. This projection may have functional significance. For example, unilateral glycine injections may inhibit the discharge of neurons located in both sides of the PRF via this pathway. This hypothesis would explain the electrophysiological finding that sympathetic responses caused by bilateral blockade of the PRF are not greater than those caused by unilateral PRF blockade (Hayes & Weaver, 1992).

To our knowledge, this study is the first to demonstrate the major efferent brainstem and spinal cord projections from PRF neurons that are involved in tonic cardiovascular regulation. Axons and terminals of PRF neurons were identified primarily within the pontine and medullary reticular formation as well as within the ventrolateral medulla. Very few PHA-L labelled fibres and terminals were observed within autonomic nuclei of the spinal cord. These results provide an anatomical substrate for tonic control of sympathetic nerve activity by pontine reticular neurons.

Summary and Conclusions

1. Inhibition of tonic activity (blockade) of neurons in the rostral ventrolateral medulla (RVLM) caused greater decreases in the discharge of postganglionic renal than that of splenic nerves and had no consistent effect on mesenteric nerves. RVLM blockade also decreased the discharge of preganglionic 13th thoracic (T_{13}) white rami more than that of preganglionic greater splanchnic nerves.

2. Ganglionic blockade with chlorisondamine decreased the discharge of severed T_{13} white rami indicating that these rami are composed of postganglionic axons (other than gray rami fibres) as well as preganglionic axons. Histological examination of excised T_{13} rami showed that postganglionic cell bodies are located along the course of these nerves.

3. Cervical (C_1) spinal cord transection caused decreases in preganglionic greater splanchnic and postganglionic splenic nerves which were of the same magnitude as those caused by bilateral blockade of the RVLM. In contrast, discharge of renal nerves was decreased more by bilateral RVLM blockade than by cervical spinal cord transection. Similarly, even unilateral RVLM blockade caused greater decreases in discharge of T_{13} white rami than were caused by spinal cord transection.

4. Inhibition of tonic activity of neurons located in the region of the A5 cell group caused increases in arterial pressure and renal and splenic nerve activity but had little effect on heart rate.

5. Inhibition of tonic activity of neurons in the pontine reticular formation (PRF) caused large, short-lasting decreases in arterial pressure and heart rate and equal

decreases in discharge of renal and splenic nerves. The short duration of the responses to PRF blockade could not be attributed to compensation by baroreceptors or by actions specific to glycine.

6. Unilateral PRF blockade eliminated the ongoing activity of 6 cardiovascular units and reduced the activity of 4 cardiovascular units located within the ipsilateral RVLM. The duration of inhibition of unit activity was not different from the duration of inhibition of renal nerve activity. PRF blockade had no effect on the discharge of 5 cardiovascular units or on the discharge of 6 non-cardiovascular units.

7. The brainstem and spinal cord projections of PRF neurons that are involved in tonic cardiovascular control were traced with the anterograde tracer *Phaseolus vulgaris leucoagglutinin*. Injections of PHA-L into the PRF identified axons and terminals primarily within the medullary reticular formation and RVLM.

These studies have focused on the control of tonic sympathetic activity by two brainstem regions, the RVLM and the PRF. Experiments have identified characteristics of sympathetic control by the RVLM which provide selective, non-uniform influences on individual organs such as the kidney, spleen and intestine. Pre- and postganglionic sympathetic pathways to the kidney appear more dependent upon excitatory drive from the RVLM than pathways directed to the spleen and intestine. Furthermore, renal nerves and their preganglionic inputs (T_{13} white rami) appear to be controlled in part by tonic sympathoinhibitory influences which can be unmasked by blockade of the RVLM. Since these sympathoinhibitory influences do not appear to

affect the activity of splanchnic and splenic nerves, tonic inhibition may be another characteristic of sympathetic control which provides differential effects on different vascular beds. Although neurons located within the region of the A5 cell group tonically inhibit sympathetic nerve activity, these neurons are not a likely source of inhibition that is selective for renal nerves.

A previously unknown source of tonic cardiovascular control in the reticular formation of the pons (PRF) has been identified in these investigations. The activity of neurons within the PRF makes a contribution to ongoing activity of sympathetic nerves supporting arterial pressure and heart rate. In addition, tonic control of sympathetic activity from the PRF differs from that from the RVLM because the PRF appears to have equal excitatory influences on discharge of both renal and splenic nerves. Furthermore, ongoing activity of PRF neurons is a source of tonic excitatory drive to some cardiovascular neurons located within the RVLM. In the absence of excitatory influences from the PRF, RVLM neurons are still capable of tonic discharge. This rapid recovery of RVLM unit activity probably explains the short duration of sympathetic responses after blockade of the PRF. PRF neurons could influence tonic sympathetic outflow by synaptic connections within the medullary reticular formation or within the RVLM.

Over the past several years, research has focused on the role of RVLM neurons in control of tonic sympathetic nerve activity. As demonstrated by the present investigations, other nuclei including the PRF also contribute to resting levels of vasomotor discharge. The importance of these other "vasomotor" regions has likely

been overlooked because destruction of these neurons fails to affect resting levels of arterial pressure in the long-term. In this respect, specific brainstem nuclei including the PRF and RVLM (Cochrane *et al.*, 1989; Vasquez *et al.*, 1992) do not appear to be crucial for ongoing vasomotor tone although both of these regions are responsible for generation of a substantial portion of tonic sympathetic activity when the neuraxis is intact. The present studies have also suggested that generation of this drive is the collective responsibility of a network of brainstem neurons that includes an interaction between the PRF and RVLM.

References

ABERCROMBIE, M. (1946). Estimation of nuclear population from microtome sections. *Anatomical Records* 94, 239-247.

ADRIAN, E.D., D.W. BRONK and G. PHILLIPS (1932). Discharges in mammalian sympathetic nerves. *Journal of Physiology* 74, 115-133.

AGARWAL, S.K. and F.R. CALARESU (1991). Monosynaptic connection from caudal to rostral ventrolateral medulla in the baroreceptor reflex pathway. *Brain Research* 555, 70-74.

AGARWAL, S.K., A.J. GELSEMA and F.R. CALARESU (1989). Neurons in rostral VLM are inhibited by chemical stimulation of caudal VLM in rats. *American Journal of Physiology* 257, R265-R270.

AGARWAL, S.K., A.J. GELSEMA and F.R. CALARESU (1990). Inhibition of rostral VLM by baroreceptor activation is relayed through caudal VLM. *American Journal of Physiology* 258, R1271-R1278.

ALEXANDER, R.S. (1946). Tonic and reflex functions of medullary sympathetic cardiovascular centers. *Journal of Neurophysiology* 9, 205-217.

AMENDT, K., J. CZACHURSKI, K. DEMBOWSKY and H. SELLER (1979). Bulbospinal projections to the intermediolateral cell column: a neuroanatomical study. *Journal of the Autonomic Nervous System* 1, 103-117.

ANDREZIK, J.A. and A.J. BEITZ (1985). Reticular formation, central gray and related tegmental nuclei. In: *The Rat Nervous System: Hindbrain and Spinal Cord*, ed. G. Paxinos. San Diego: Academic Press Inc., pp. 1-28.

ANDREZIK, J.A., V. CHAN-PALAY and S.L. PALAY (1981). The nucleus paragigantocellularis lateralis in the rat: conformation and cytology. *Anatomy and Embryology* 161, 355-371.

APRISON, M.H. and E.C. DALY (1978). Biochemical aspects of transmission at inhibitory synapses: the role of glycine. *Advances in Neurochemistry* 3, 203-294.

ARDELL, J.L., S.M. BARMAN and G.L. GEBBER (1982). Sympathetic nerve discharge in chronic spinal cat. *American Journal of Physiology* 243, H463-H470.

ASFOURY, Z.M. *Sympathectomy and the Innervation of the Kidney*, New York: Appleton-Century-Crofts, 1971, pp. 73-76.

BACON, S.J., A. ZAGON and A.D. SMITH (1990). Electron microscopic evidence of a monosynaptic pathway between cells in the caudal raphe nuclei and sympathetic preganglionic neurons in the rat spinal cord. *Experimental Brain Research* 79, 589-602.

BALJET, B. and J. DRUKKER (1979). The extrinsic innervation of the abdominal organs in the female rat. *Acta Anatomica* 104, 243-267.

BARMAN, S.M. (1990) Brainstem control of cardiovascular function. In: *Brainstem Mechanisms of Behaviour*, eds. W.R. Klemm and Robert P. Vertes. John Wiley & Sons Inc., pp. 353-381.

BARMAN, S.M. and G.L. GEBBER (1978). Tonic sympathoinhibition in the baroreceptor denervated cat. *Proceedings of the Society of Experimental Biology* 157, 648-655.

BARMAN, S.M. and G.L. GEBBER (1981). Problems associated with the identification of brain stem neurons responsible for sympathetic nerve discharge. *Journal of the Autonomic Nervous System* 3, 369-377.

BARMAN, S.M. and G.L. GEBBER (1983). Sequence of activation of ventrolateral and dorsal medullary sympathetic neurons. *American Journal of Physiology* 245, R438-R447.

BARMAN, S.M. and G.L. GEBBER (1985). Axonal projection patterns of ventrolateral medullospinal sympathoexcitatory neurons. *Journal of Neurophysiology* 53 (6), 1551-1566.

BARMAN, S.M. and G.L. GEBBER (1987). Lateral tegmental field neurons of cat medulla: a source of basal activity of ventrolateral medullospinal sympathoexcitatory neurons. *Journal of Neurophysiology* 57(5), 1410-1424.

BARMAN, S.M. and G.L. GEBBER (1988). The axons of raphespinal sympathoinhibitory neurons branch in the cervical spinal cord. *Brain Research* 441, 371-376.

BARMAN, S.M. and G.L. GEBBER (1989). Lateral tegmental field neurons of cat medulla: a source of basal activity of raphespinal sympathoinhibitory neurons. *Journal of Neurophysiology* 61(5), 1011-1024.

BARMAN, S.M., G.L. GEBBER and F.R. CALARESU (1984). Differential control of sympathetic nerve discharge by the brain stem. *American Journal of Physiology* 247, R513-R519.

BARON, R.B.W., W. JANIG and E.M. MCLACHLAN (1985). The afferent and sympathetic components of the lumbar spinal outflow to the colon and pelvic organs in the cat. II. the lumbar splanchnic nerves *Journal of Comparative Neurology* 238, 147-157.

BARRÉS, C.P., S.J. LEWIS, C.L. GROSSKREUTZ, K.J. VARNER and M.J. BRODY (1989). Role of renal nerves in experimental hypertension: evaluation of neurogenic mechanisms. *Clinical and Experimental Hypertension* A11(Suppl.1), 117-124.

BELULI, D.J. and L.C. WEAVER (1991a). Differential control of renal and splenic nerves without medullary topography. *American Journal of Physiology* 260, H1072-H1079.

BELULI, D.J. and L.C. WEAVER (1991b). Areas of rostral medulla providing tonic control of renal and splenic nerves. *American Journal of Physiology* 261, H1687-H1692.

BETZ, H. and C.-M. BECKER (1988). The mammalian glycine receptor: biology and structure of a neuronal chloride channel protein. *Neurochemistry International* 13, 137-146.

BLAIR, R.W. (1987). Responses of feline medial medullary reticulospinal neurons to cardiac input. *Journal of Neurophysiology* 58(5), 1149-1167.

BLESSING, W.W. (1988). Depressor neurons in rabbit caudal medulla act via GABA receptors in rostral medulla. *American Journal of Physiology* 254, H686-H692.

BLESSING, W.W. (1990). Distribution of glutamate decarboxylase-containing neurons in rabbit medulla oblongata with attention to intramedullary and spinal projections. *Neuroscience* 37(1), 171-185.

BLESSING, W.W., A.K. GOODCHILD, R.A.L. DAMPNEY and J.P. CHALMERS (1981b). Cell groups in the lower brain stem of the rabbit projecting to the spinal cord, with special reference to catecholamine-containing neurons. *Brain Research* 221, 35-55.

BLESSING, W.W. and Y.-W. Li (1989). Inhibitory vasomotor neurons in the caudal ventrolateral region of the medulla oblongata. In: *Progress in Brain Research*, eds. J. Ciriello, M.M. Caverson and C. Polosa., Amsterdam: Elsevier Science Publishers, pp. 83-97.

BLESSING, W.W., J.R. OLIVER, A.H. HODGSON, T.H. JOH and J.O. WILLOUGHBY (1987). Neuropeptide Y-like immunoreactive C1 neurons in the rostral ventrolateral medulla of the rabbit project to sympathetic preganglionic neurons in the spinal cord. *Journal of the Autonomic Nervous System* 18, 121-129.

BLESSING, W.W. and D.J. REIS (1982). Inhibitory cardiovascular function of neurons in the caudal ventrolateral medulla of the rabbit: relationship to the area containing A1 noradrenergic cells. *Brain Research* 253, 161-171.

BLESSING, W.W., A.F. SVED and D.J. REIS (1984). Arterial pressure and plasma vasopressin: regulation by neurons in the caudal ventrolateral medulla. *Clinical and Experimental Hypertension* A6(1&2), 149-156.

BLESSING, W.W., M.J. WEST and J.P. CHALMERS (1981a). Hypertension, bradycardia, and pulmonary edema in the conscious rabbit after brainstem lesions coinciding with the A1 group of catecholamine neurons. *Circulation Research* 49, 949-958.

BOBILLIER, P., S. SEGUIN, F. PETITJEAN, D. SALVERT, M. TOURET and M. JOUVET (1976). The raphe nuclei of the cat brain stem: a topographical atlas of their efferent projections as revealed by autoradiography. *Brain Research* 113, 449-486.

BOYD, J.D. (1957). Intermediate sympathetic ganglia. *British Medical Bulletin* 13, 207-212.

BRENDER, D. and M.M. WEBB-PEPLOE (1969). Influence of carotid baroreceptors on different components of the vascular system. *Journal of Physiology* 205, 257-274.

BROOKS-FOURNIER, R. and R.E. COGGESHALL (1981). The ratio of preganglionic axons to postganglionic cells in the sympathetic nervous system of the rat. *Journal of Comparative Neurology* 197, 207-216.

BROOKSBY, G.A. and D.E. DONALD (1971). Dynamic changes in splanchnic blood flow and blood volume in dogs during activation of sympathetic nerves. *Circulation Research* 29(3), 227-238.

BROWN, D.L. and P.G. GUYENET (1984). Cardiovascular neurons of brain stem with projections to spinal cord. *American Journal of Physiology* 247, R1009-R1016.

BROWN, D.L. and P.G. GUYENET (1985). Electrophysiological study of cardiovascular neurons in the rostral ventrolateral medulla in rats. *Circulation Research* 56, 359-369.

BYRUM, C.E. and P.G. GUYENET (1987). Afferent and efferent connections of the A5 noradrenergic cell group in the rat. *Journal of Comparative Neurology* 261, 529-542.

BYRUM, C.E., R. STORNETTA and P.G. GUYENET (1984). Electrophysiological properties of spinally-projecting A5 noradrenergic neurons. *Brain Research* 303, 15-29.

CABOT, J.B. (1990). Sympathetic preganglionic neurons: cytoarchitecture, ultrastructure, and biophysical properties. In: *Central Regulation of Autonomic Functions*, eds. A.D. Loewy and K.M. Spyer. New York: Oxford University Press, pp. 44-67.

CABOT, J.B., J.M. WILD and D.H. COHEN (1979). Raphe inhibition of sympathetic preganglionic neurons. *Science* 203, 184-186.

CALARESU, F.R., J.C. TOBEY, S.R. HEIDEMANN and L.C. WEAVER (1984). Splenic and renal sympathetic responses to stimulation of splenic receptors in cats. *American Journal of Physiology* 247, R856-R865.

CALARESU, F.R. and C.P. YARDLEY (1988). Medullary basal sympathetic tone. *Annual Reviews of Physiology* 50, 511-524.

CANNON, W.B. (1929). The sympathetic division of the autonomic system in relation to homeostasis. *Archives of Neurology and Psychiatry* 22, 282-294.

CARLTON, S.M., J.M. CHUNG, R.B. LEONARD and W.D. WILLIS (1985). Funicular trajectories of brainstem neurons projecting to the lumbar spinal cord in the monkey (Macaca fascicularis): a retrograde labelling study. *Journal of Comparative Neurology* 241, 382-404.

CARNEIRO, J.J. and D.E. DONALD (1977). Blood reservoir function of dog spleen, liver, and intestine. *American Journal of Physiology* 1(1), H67-H72.

CAVERSON, M.M., J. CIRIELLO and F.R. CALARESU (1983). Direct pathway from cardiovascular neurons in the ventrolateral medulla to the region of the intermediolateral nucleus of the upper thoracic cord: an anatomical and electrophysiological investigation in the cat. *Journal of the Autonomic Nervous System* 9, 451-475.

CELLER, B.G. and L.P. SCHRAMM (1981). Pre- and postganglionic sympathetic activity in splanchnic nerves of rats. *American Journal of Physiology* 241, R55-R61.

CHAI, C.Y., R.H. LIN, A.M.Y. LIN, C.M. PAN, E.H.Y. LEE and J.S. KUO (1988). Pressor responses from electrical or glutamate stimulations of the dorsal or ventrolateral medulla. *American Journal of Physiology* 255, R709-R717.

CHAI, C.Y. and S.C. WANG (1968). Integration of sympathetic cardiovascular mechanisms in medulla oblongata of the cat. *American Journal of Physiology* 215(6), 1310-1315.

CHALMERS, J.P. and P.M. PILOWSKY (1991). Brainstem and bulbospinal neurotransmitter systems in the control of blood pressure. *Journal of Hypertension* 9, 675-694.

CHAN, R.K.W., Y.S. CHAN and T.M. WONG (1991). Electrophysiological properties of neurons in the rostral ventrolateral medulla of normotensive and spontaneously hypertensive rats. *Brain Research* 549, 118-126.

CHEVENDRA, V. and L.C. WEAVER (1991). Distribution of splenic, mesenteric and renal neurons in sympathetic ganglia in rats. *Journal of the Autonomic Nervous System* 33, 47-54.

CHRONWALL, B.M., D.A. DI MAGGIO, V.J. MASSARI, V.M. PICKEL, D.A. RUGGIERO and T.L. O'DONOHUE (1985). The anatomy of neuropeptide-Y - containing neurons in rat brain. *Neuroscience* 15(4), 1159-1181.

CIRIELLO, J., M.M. CAVERSON and C. POLOSA (1986). Function of the ventrolateral medulla in the control of the circulation. *Brain Research Bulletin* 11, 359-391.

CLOSE, J.M., J.J. NEIL and A.D. LOEWY (1982). Actions of N-methyl aspartate and its antagonist aminophosphonovalerate on the A5 catecholamine cell group in rat. *Brain Research* 249, 393-396.

COCHRANE, K.L., R.A. BUCHHOLZ, J.W. HUBBARD, T.K. KEETON and M.A. NATHAN (1988). Hypotensive effects of lesions of the rostral ventrolateral medulla in rats are anesthetic-dependent. *Journal of the Autonomic Nervous System* 22, 181-187.

COCHRANE, K.L. and M.A. NATHAN (1989). Normotension in conscious rats after placement of bilateral electrolytic lesions in the rostral ventrolateral medulla. *Journal of the Autonomic Nervous System* 26, 199-211.

COOTE, J.H. (1985). Noradrenergic projections to the spinal cord and their role in cardiovascular control. *Journal of the Autonomic Nervous System* 14, 255-262.

COOTE, J.H. and V.H. MACLEOD (1974). The influence of bulbospinal monoaminergic pathways on sympathetic nerve activity. *Journal of Physiology* 241, 453-475.

COOTE, J.H. and V.H. MACLEOD (1975). The spinal route of sympatho-inhibitory pathways descending from the medulla oblongata. *Pflugers Archives* 359, 335-347.

COOTE, J.H., V.H. MACLEOD, S.M. FLEETWOOD-WALKER and M.P. GILBEY (1981). The response of individual sympathetic preganglionic neurones to microelectrophoretically applied endogenous monoamines. *Brain Research* 215, 135-145.

COOTE, J.H. and D.R. WESTBURY (1979). Intracellular recordings from sympathetic preganglionic neurones. *Neuroscience Letters* 15, 171-175.

COSTA, M. and J.B. FURNESS (1984). Somatostatin is present in a subpopulation of noradrenergic nerve fibres supplying the intestine. *Neuroscience* 13(3), 911-919.

DAHLSTROM, A. and K. FUXE (1964). Evidence for the existence of monoamine-containing neurons in the central nervous system. *Acta Physiologica Scandinavia* 62 (suppl.232), 1-55.

DAMPNEY, R.A.L., W.W. BLESSING and E. TAN (1988). Origin of tonic GABAergic inputs to vasopressor neurons in the subretrofacial nucleus of the rabbit. *Journal of the Autonomic Nervous System* 24, 227-239.

DAMPNEY, R.A.L. and R.M. MCALLEN (1988). Differential control of sympathetic fibres supplying hindlimb skin and muscle by subretrofacial neurones in the cat. *Journal of Physiology* 395, 41-56.

DAMPNEY, R.A.L. and E.A. MOON (1980). Role of ventrolateral medulla in vasomotor response to cerebral ischemia. *American Journal of Physiology* 239, H349-H358.

DE GROAT, W.C. and P.M. LALLEY (1974). Reflex sympathetic firing in response to electrical stimulation of the carotid sinus nerve in the cat. *Brain Research* 80, 17-40.

DEAN, C. and J.H. COOTE (1986). A ventromedullary relay involved in the hypothalamic and chemoreceptor activation of sympathetic postganglionic neurones to skeletal muscle, kidney and splanchnic area. *Brain Research* 377, 279-285.

DEFEUDIS, F. (1990). Brainstem systems involved in cardiovascular regulation. *Annals of the New York Academy of Science.* 585, 231-240.

DEHAL, N.S., G.A. DEKABAN, A.V. KRASSIOUKOV, F.J. PICARD and L.C. WEAVER (1992). Identification of renal preganglionic neurons in hamsters using transynaptic transport of herpes simplex type 1 virus. *Neuroscience*, (submitted).

DEMBOWSKY, K., J. CZACHURSKI, K. AMENDT and H. SELLER (1980). Tonic descending inhibition of the spinal somato-sympathetic reflex from the lower brain stem. *Journal of the Autonomic Nervous System* 2, 157-182.

DEMBOWSKY, K., J. CZACHURSKI and H. SELLER (1985). An intracellular study of the synaptic input to sympathetic preganglionic neurones of the third thoracic segment of the cat. *Journal of the Autonomic Nervous System* 13, 201-244.

DEMBOWSKY, K., J. CZACHURSKI and H. SELLER (1989). Some properties of the sympathoinhibition from the caudal ventrolateral medulla oblongata in the cat. *Progress in Brain Research* 81, 143-157.

DEMBOWSKY, K. and R.M. MCALLEN (1990). Baroreceptor inhibition of subretrofacial neurons: evidence from intracellular recordings in the cat. *Neuroscience Letters* 111, 139-143.

DORMER, K.J., J.A. ANDREZIK, R.J. PERSON, J.T. BRAGGIO and R.D. FOREMAN (1986). Fastigial nucleus cardiovascular response and brainstem lesions in the beagle. *American Journal of Physiology* 250, H231-H239.

DORWARD, P.K., S.L. BURKE, W. JANIG and J. CASSELL (1987). Reflex responses to baroreceptor, chemoreceptor and nociceptor inputs in single renal sympathetic neurones in the rabbit and the effects of anaesthesia on them. *Journal of the Autonomic Nervous System* 18, 39-54.

FELDBERG, W. and P.G. GUERTZENSTEIN (1972). A vasodepressor effect of pentobarbitone sodium. *Journal of Physiology* 224, 83-103.

FELDBERG, W. and P.G. GUERTZENSTEIN (1976). Vasodepressor effects obtained by drugs acting on the ventral surface of the brainstem. *Journal of Physiology*, 258, 337-355.

FELDMAN, J.L. and H.H. ELLENBERGER (1988). Central coordination of respiratory and cardiovascular control in mammals. *Annual Reviews of Physiology* 50, 5[illegible]3-606.

FERGUSON. M., G.B. RYAN and C. BELL (1986). Localization of sympathetic and sensory neurons innervating the rat kidney. *Journal of the Autonomic Nervous System* 16, 279-288.

FINLEY, J.C.W., J.L. MADERDRUT, L.J. ROGER and P. PETRUSZ (1981). The immunocytochemical localization of somatostatin-containing neurons in the rat central nervous system. *Neuroscience* 6(11), 2173-2192.

FLEETWOOD-WALKER, S.M., J.H. COOTE and M.P. GILBEY (1983). Identification of spinally projecting neurones in the A1 catecholamine cell group of the ventrolateral medulla. *Brain Research* 273, 25-33.

FOLEY, J.O. (1945). The components of the cervical sympathetic trunk with special reference to its accessory cells and ganglia. *Journal of Comparative Neurology* 82, 77-91.

FOREMAN, R.D. and R.W. BLAIR (1988). Central organization of sympathetic cardiovascular response to pain. *Annual Reviews of Physiology* 50, 607-622.

FULWILER, C.E. and C.B. SAPER (1984). Subnuclear organization of the efferent connections of the parabrachial nucleus in the rat. *Brain Research Reviews* 7, 229-259.

FUTURO-NETO, H.A. and J.H. COOTE (1982). Desynchronized sleep-like pattern of sympathetic activity elicited by electrical stimulation of sites in the brain. *Brain Research* 252, 269-276.

GATTI, P.J. and R.A. GILLIS (1991). Interaction between pressor and depressor areas in cat ventrolateral medulla. *Brain Research* 552, 153-158.

GEBBER, G.L. (1984). Brainstem systems involved in cardiovascular regulation. In: *Nervous Control of Cardiovascular Function*, ed. W.C. Randall. New York: Oxford University Press, pp. 346-368.

GEBBER, G.L. (1990). Central determinants of sympathetic nerve discharge. In: *Central Regulation of Autonomic Function*, eds. A.D. Loewy and K.M. Spyer. New York: Oxford University Press, pp. 126-144.

GEBBER, G.L. and S.M. BARMAN (1985). Lateral tegmental field neurons of cat medulla: a potential source of basal sympathetic nerve discharge. *Journal of Neurophysiology* 54(6), 1498-1512.

GEBBER, G.L. and S.M. BARMAN (1988). Studies on the origin and generation of sympathetic nerve activity. *Clinical and Experimental Hypertension* A10 (Suppl. 1), 33-44.

GERFEN, C.R. and P.E. SAWCHENKO (1984). An anterograde neuroanatomical tracing method that shows the detailed morphology of neurons, their axons and terminals: immunohistochemical localization of an axonally transported plant lectin, Phaseolus vulgaris leucoagglutinin (PHA-L). *Brain Research* 290, 219-238.

GILBEY, M.P., J.H. COOTE, V.H. MACLEOD and D.F. PETERSON (1981). Inhibition of sympathetic activity by stimulating in the raphe nuclei and the role of 5-hydroxytryptamine in this effect. *Brain Research* 226, 131-142.

GOODCHILD, A.K., R.A.L. DAMPNEY and R. BANDLER (1982). A method for evoking physiological responses by stimulation of cell bodies, but not axons of passage, within localized regions of the central nervous system. *Journal of Neuroscience Methods* 6, 351-363.

GORDON, F.J. (1987). Aortic baroreceptor reflexes are mediated by NMDA receptors in caudal ventrolateral medulla. *American Journal of Physiology* 252, R628-R633.

GRANATA, A.R., M. KUMADA and D.J. REIS (1985b). Sympathoinhibition by A1-noradrenergic neurons is mediated by neurons in the C1 area of the rostral medulla. *Journal of the Autonomic Nervous System* 14, 387-395.

GRANATA, A.R., Y. NUMAO, M. KUMADA and D.J. REIS (1986). A1 noradrenergic neurons tonically inhibit sympathoexcitatory neurons of C1 area in rat brainstem. *Brain Research* 377, 127-146.

GRANATA, A.R., D.A. RUGGIERO, D.H. PARK, T.H. JOH and D.J. REIS (1985a). Brain stem area with C1 epinephrine neurons mediates baroreflex vasodepressor responses. *American Journal of Physiology* 248, H547-H567.

GREENWAY, C.V. and G.E. LISTER (1974). Capacitance effects and blood reservoir function in the splanchnic vascular bed during non-hypotensive haemorrhage and blood volume expansion in anaesthetized cats. *Journal of Physiology* 237, 279-294.

GUERTZENSTEIN, P.G. (1973). Blood pressure effects obtained by drugs applied to the ventral surface of the brain stem. *Journal of Physiology* 229, 395-408.

GUERTZENSTEIN, P.G. and A. SILVER (1974). Fall in blood pressure produced from discrete regions of the ventral surface of the medulla by glycine and lesions. *Journal of Physiology* 242, 489-503.

GURTU, S., K.K. PANT, J.N. SINHA and K.P. BHARGAVA (1984). An investigation into the mechanism of cardiovascular responses elicited by electrical stimulation of locus coeruleus and subcoeruleus in the cat. *Brain Research* 301, 59-64.

GUYENET, P.G. (1990). Role of the ventral medulla oblongata in blood pressure regulation. In: *Central Regulation of Autonomic Functions*, eds. A.D. Loewy and K.M. Spyer. New York: Oxford University Press, pp. 145-167.

GUYENET, P.G., M.-K. SUN and D.L. BROWN. (1987). Role of GABA and excitatory amino acids in medullary baroreflex pathways. In: *Organization of the Autonomic Nervous System: Central and Peripheral Mechanisms*, eds. J. Ciriello, F.R. Calaresu, L.P. Renaud and C. Polosa. New York: Alan R. Liss, Inc., pp. 215-225.

GUYENET, P.G. and J.B. CABOT (1981). Inhibition of sympathetic preganglionic neurons by catecholamines and clonidine: mediation by an adrenergic receptor. *Journal of Neuroscience* 1, 908-917.

HAYES, K., D.J. BELULI and L.C. WEAVER (1990b). Tonic sympathetic control of the kidney, spleen and heart originating from ventrolateral pontine neurons in rats. *Society for Neuroscience Abstracts* 16, 214.

HAYES, K., F.R. CALARESU and L.C. WEAVER (1993). Pontine reticular neurons provide tonic excitation to neurons in the rostral ventrolateral medulla in rats. *American Journal of Physiology*, (In Press).

HAYES, K., V. CHEVENDRA and L.C. WEAVER (1990a). Pre- and postganglionic sympathetic activity in white rami of rats. *Neuroscience Letters* 115, 55-61.

HAYES, K. and L.C. WEAVER (1990). Selective control of sympathetic pathways to the kidney, spleen and intestine by thc ventrolateral medulla in rats. *Journal of Physiology* 428, 371-385.

HAYES, K. and L.C. WEAVER (1992). Tonic sympathetic excitation and vasomotor control from pontine reticular neurons. *American Journal of Physiology* 263, H1567-H1575.

HERBERT, H., M.M. MOGA and C.B. SAPER (1990). Connections of the parabrachial nucleus with the nucleus of the solitary tract and the medullary reticular formation in the rat. *Journal of Comparative Neurology* 293, 540-580.

HILTON, S.M. (1975). Ways of viewing the central nervous control of the circulation - old and new. *Brain Research* 87, 213-219.

HILTON, S.M. (1979). The defense reaction as a paradigm for cardiovascular control. In: *Integrative Functions of the Autonomic Nervous System*, eds. C.M. Brooks, K. Koizumi and A. Sato. Amsterdam: University of Tokyo Press, pp. 441-449.

HILTON, S.M. and K.M. SPYER (1980). Central nervous system regulation of vascular resistance. *Annual Reviews of Physiology* 42, 399-411.

HOKFELT, T., J.M. LUNDBERG, K. TATEMOTO, V. MUTT, L. TERENIUS, J. POLAK, S. BLOOM, C. SASEK, R. ELDE and M. GOLDSTEIN (1983). Neuropeptide Y (NPY)- and FMRFamide neuropeptide-like immunoreactivities in catecholamine neurons of the rat medulla oblongata. *Acta Physiologica Scandinavia* 117, 315-318.

HOLSTEGE, G., D. GRIFFITHS, H. DE WALL and E. DALM (1986). Anatomical and physiological observations on supraspinal control of bladder and urethral sphincter muscles in the cat. *Journal of Comparative Neurology* 250, 449-461.

HUANG, Z.-S., G.L. GEBBER, S.M. BARMAN and K.J. VARNER (1987). Forebrain contribution to sympathetic nerve discharge in anesthetized cats. *American Journal of Physiology* 252, R645-R652.

HUANG, Z.-S., K.J. VARNER, S.M. BARMAN and G.L. GEBBER (1988). Diencephalic regions contributing to sympathetic nerve discharge in anesthetized cats. *American Journal of Physiology* 254, R249-R256.

HUANGFU, D., L.-J. HWANG, T.A. RILEY and P.G. GUYENET (1992). Splanchnic nerve response to A5 area stimulation in rats. *American Journal of Physiology* 263, R437-R446.

HUANGFU, D., N. KOSHIYA and P.G. GUYENET (1991). A5 noradrenergic unit activity and sympathetic nerve discharge in rats. *American Journal of Physiology* 261, R393-R402.

HUDSON, M.E., K. FUXE, M. GOLDSTEIN and M. KALIA (1986). Spinal projections of noradrenergic and adrenergic neurons in the medulla oblongata: new evidence in central cardiovascular control. *Society for Neuroscience Abstracts* 12(1), 535.

IRIUCHIJIMA, J. and S. SAKATA (1985). Regional difference in sympathetic vasoconstrictor tone in conscious rats. *Japanese Journal of Physiology* 35, 1051-1063.

ISHIKAWA, N., C.H. KALLMAN and K. SAGAWA (1984). Rabbit carotid sinus reflex under pentobarbital, urethan, and chloralose anesthesia. *American Journal of Physiology* 246, H696-H701.

KENNEY, M.J., S.M. BARMAN and G.L. GEBBER (1989). Anesthetic-dependent contributions of rostral lateral tegmental field (rLTF) neurons to sympathetic nerve discharge (SND). *Society for Neuroscience Abstracts* 15, 812.

KIHARA, M. and T. KUBO (1989). Immunocytochemical localization of GABA containing neurons in the ventrolateral medulla oblongata of the rat. *Histochemistry* 91, 309-314.

KORKOLA, M.L. and L.C. WEAVER (1992). Role of dorsal medullary reticular formation in maintenance of vasomotor tone in rats. *American Journal of Physiology*, (submitted).

KRASSIOUKOV, A.V., A.W. GELB and L.C. WEAVER (1992). Action of propofol on central sympathetic mechanisms controlling blood pressure. *Anaesthesiology*, (submitted).

KRASSIOUKOV, A.V. and L.C. WEAVER (1992). Connections between the pontine reticular formation and rostral ventrolateral medulla. *American Journal of Physiology*, (submitted).

KRUKOFF, T.L., T. VU, K.H. HARRIS, S. AIPPERSBACH and J.H. JHAMANDAS (1992). Neurons in the rat medulla oblongata containing neuropeptide Y-, angiotensin II-, or galanin-like immunoreactivity project to the parabrachial nucleus. *Neuroscience* 47(1), 175-184.

KUMADA, M., R.A.L. DAMPNEY and D.J. REIS (1979). Profound hypotension and abolition of the vasomotor component of the cerebral ischemic response produced by restricted lesions of medulla oblongata in rabbit. *Circulation Research* 45(1), 63-70.

KUO, D.C., G.C.H. YANG, D.S. YAMASAKI and G.M. KRAUTHAMER (1982). A wide field electron microscopic analysis of the fiber constituents of the major splanchnic nerve in cat. *Journal of Comparative Neurology* 210, 49-58.

LAI, Y.Y. and J.M. SIEGEL (1990). Cardiovascular and muscle tone changes produced by microinjection of cholinergic and glutamatergic agonists in dorsolateral pons and medial medulla. *Brain Research* 514, 27-36.

LEIBSTEIN, A.G., R. DERMIETZEL, I.M. WILLENBERG and R. PAUSCHERT (1985). Mapping of different neuropeptides in the lower brainstem of the rat: with special reference to the ventral surface. *Journal of the Autonomic Nervous System* 14, 299-313.

LI, Y.-W., Z.-Q. DING, S.L. WESSELINGH and W.W. BLESSING (1992a). Renal and adrenal sympathetic preganglionic neurons in rabbit spinal cord: tracing with Herpes simplex virus. *Brain Research* 573, 147-152.

LI, Y.-W., Z.J. GIEROBA, R.M. MCALLEN and W.W. BLESSING (1991). Neurons in rabbit caudal ventrolateral medulla inhibit bulbospinal barosensitive neurons in rostral medulla. *American Journal of Physiology* 261, R44-R51.

LI, Y.-W., S.L. WESSELINGH and W.W. BLESSING (1992b). Projections from rabbit caudal medulla to C1 and A5 sympathetic premotor neurons, demonstrated with phaseolus leucoagglutinin and herpes simplex virus. *Journal of Comparative Neurology* 317, 379-395.

LIPSKI, J., M.C. BELLINGHAM, M.J. WEST and P.M. PILOWSKY (1988). Limitations of the technique of pressure microinjection of excitatory amino acids for evoking responses from localized regions of the CNS. *Journal of the Autonomic Nervous System* 26, 169-179.

LOEWY, A.D. (1981). Raphe pallidus and raphe obscurus projections to the intermediolateral cell column in the rat. *Brain Research* 222, 129-133.

LOEWY, A.D. (1987). Substance P neurons of the ventral medulla: their role in the control of vasomotor tone. In: *Cardiogenic Reflexes*, eds. R. Hainsworth, R.J. Linden, P.N. McWilliam and D.A.S.G. Mary. Oxford: Oxford University Press, pp. 269-285.

LOEWY, A.D., L. MARSON, D. PARKINSON, M.A. PERRY and W.B. SAWYER (1986). Descending noradrenergic pathways involved in the A5 depressor response. *Brain Research* 386, 313-324.

LOEWY, A.D. and S. MCKELLAR (1981). Serotonergic projections from the ventral medulla to the intermediolateral cell column in the rat. *Brain Research* 211, 146-152.

LOEWY, A.D., S. MCKELLAR and C.B. SAPER (1979a). Direct projections from the A5 catecholamine cell group to the intermediolateral cell column. *Brain Research* 174, 309-314.

LOEWY, A.D., C.B. SAPER and R.P. BAKER (1979b). Descending projections from the pontine micturition center. *Brain Research* 172, 533-538.

LOVICK, T.A. (1987). Differential control of cardiac and vasomotor activity by neurones in nucleus paragigantocellularis lateralis in the cat. *Journal of Physiology* 389, 23-35.

LUKOVIC, L. and W. DE JONG (1990). Analysis of the cardiovascular changes evoked by microinjection of NaCl into the nucleus tractus solitarii of rats: evidence for a Na+/Ca2+ relationship. *Neuroscience Letters* 110, 46-51.

MAASS-MORENO, R. and C.F. ROTHE (1991). Carotid baroreceptor control of liver and spleen volume in cats. *American Journal of Physiology* 260, H254-H259.

MALMSTADT, H., C. ENKE and S. CROUCH. (1981). *Electronics and Instrumentation for Scientists*. Menlo,CA: The Benjamin/Cummings Publishing Company, Inc., pp. 1-408.

MANTYH, P.W. and S.P. HUNT (1984). Evidence for cholecystokinin-like immunoreactive neurons in the rat medulla oblongata which project to the spinal cord. *Brain Research* 291, 49-54.

MASUDA, N., N. TERUI, N. KOSHIYA and M. KUMADA (1991). Neurons in the caudal ventrolateral medulla mediate the arterial baroreceptor reflex by inhibiting barosensitive reticulospinal neurons in the rostral ventrolateral medulla in rabbits. *Journal of the Autonomic Nervous System* 34, 103-118.

MCALLEN, R.M. (1986). Action and specificity of ventral medullary vasopressor neurones in the cat. *Neuroscience* 18(1), 51-59.

MCALLEN, R.M. and R.A.L. DAMPNEY (1990). Vasomotor neurons in the rostral ventrolateral medulla are organized topographically with respect to type of vascular bed but not body region. *Neuroscience Letters* 110, 91-96.

MCCALL, R.B. (1988b). Effects of neurotransmitters on sympathetic preganglionic neurons. *Annual Reviews of Physiology* 50, 553-564.

MCCALL, R.B. (1988a). GABA-mediated inhibition of sympathoexcitatory neurons by midline medullary stimulation. *American Journal of Physiology* 255, R605-R615.

MCCALL, R.B. (1990). Central neurotransmitters involved in cardiovascular regulation. In: *Cardiovascular Pharmacology*, ed. M. Antonaccio. New York: Raven Press Ltd., pp. 161-200.

MCCALL, R.B. and M.E. CLEMENT (1989). Identification of serotonergic and sympathetic neurons in medullary raphe nuclei. *Brain Research* 477, 172-182.

MCCALL, R.B. and L.T. HARRIS (1987). Sympathetic alterations after midline medullary raphe lesions. *American Journal of Physiology* 253, R91-R100.

MCCALL, R.B. and S.J. HUMPHREY (1985). Evidence for GABA mediation of sympathetic inhibition evoked from midline medullary depressor sites. *Brain Research* 339, 356-360.

MCCARLEY, R.W. (1980). Mechanisms and models of behavioral state control-chairman's overview of part V. In: *The Reticular Formation Revisited*. New York: Raven, pp. 375-403.

MCLACHLAN, E.M. (1974). The formation synapses mammalian sympathetic ganglia reinnervated with preganglionic or somatic nerves. *Journal of Physiology* 237, 217-242.

MECKLER, R.L. and L.C. WEAVER (1985). Splenic, renal and cardiac nerves have unequal dependence upon tonic supraspinal inputs. *Brain Research* 338, 123-135.

MECKLER, R.L. and L.C. WEAVER (1988). Characteristics of ongoing and reflex discharge of single splenic and renal sympathetic postganglionic fibres in cats. *Journal of Physiology* 396, 139-153.

MELANDER, T., T. HOKFELT, A. ROKAEUS, A.C. CUELLO, W.H. OERTEL, A. VERHOFSTAD and M. GOLDSTEIN (1986). Coexistence of galanin-like immunoreactivity with catecholamines, 5-hydroxytryptamine, GABA and neuropeptides in the rat CNS. *Journal of Neuroscience* 6(12), 3640-3654.

MILLER, J.F. and H.K. PROUDFIT (1990). Antagonism of stimulation-produced antinociception from ventrolateral pontine sites by intrathecal administration of alpha-adrenergic antagonists and naloxone. *Brain Research* 530, 20-34.

MILNER, T.A., V.M. PICKEL, R. GIULIANO and D.J. REIS (1989). Ultrastructural localization of choline acetyltransferase in the rat rostral ventrolateral medulla: evidence for major synaptic relations with non-catecholaminergic neurons. *Brain Research* 500, 67-89.

MINKELS, R.F., P.J.W. JÖCH, G.J. TER HORST and J.D. VAN WILLIGEN (1991). Projections of the parvocellular reticular formation to the contralateral mesencephalic trigeminal nucleus in the rat. *Brain Research* 547, 13-21.

MINSON, J.B, P.M. PILOWSKY, I. LLEWELLYN-SMITH, T. KANEKO, V. KAPOOR and J.P. CHALMERS (1991). Glutamate in spinally projecting neurons of the rostral ventral medulla. *Brain Research* 555, 326-331.

MINSON, J.B., J.P. CHALMERS, A.C. CAON and B. RENAUD (1987). Separate areas of rat medulla oblongata with populations of serotonin- and adrenaline-containing neurons alter blood pressure after L-glutamate stimulation. *Journal of the Autonomic Nervous System* 19, 39-50.

MIRGORODSKY, V.N. and V.I. SKOK (1969). Intracellular potentials recorded from a tonically active mammalian sympathetic ganglion. *Brain Research* 15, 570-572.

MIURA, M. and K. TAKAYAMA (1991). Circulatory and respiratory responses to glutamate stimulation of the lateral parabrachial nucleus of the cat. *Journal of the Autonomic Nervous System* 32, 121-134.

MORRISON, S.F., P. ERNSBERGER, T.A. MILNER, J. CALLAWAY, A. GONG and D.J. REIS (1989). A glutamate mechanism in the intermediolateral nucleus mediates sympathoexcitatory responses to stimulation of the rostral ventrolateral medulla. In: *Progress in Brain Research*, eds. J. Ciriello, M.M. Caverson and C. Polosa. Amsterdam: Elsevier Science Publishers B.V. (Biomedical Division), pp. 159-169.

MORRISON, S.F. and G.L. GEBBER (1982). Classification of raphe neurons with cardiac-related activity. *American Journal of Physiology* 243, R49-R59.

MORRISON, S.F. and G.L. GEBBER (1984). Raphe neurons with sympathetic-related activity: baroreceptor responses and spinal connections. *American Journal of Physiology* 246, R338-R348.

MORRISON, S.F. and G.L. GEBBER (1985). Axonal branching patterns and funicular trajectories of raphespinal sympathoinhibitory neurons. *Journal of Neurophysiology* 53(3), 759-771.

MORRISON, S.F., T.A. MILNER and D.J. REIS (1988). Reticulospinal vasomotor neurons of the rat rostral ventrolateral medulla: relationship to sympathetic nerve activity and the C1 adrenergic cell group. *Journal of Neuroscience* 8(4), 1286-1301.

MORRISON, S.F. and D. WHITEHORN (1984). Enhanced preganglionic sympathetic nerve responses in spontaneously hypertensive rats. *Brain Research* 296, 152-155.

MORROW, T.J. and K.L. CASEY (1983). Suppression of bulboreticular unit responses to noxious stimuli by analgesic mesencephalic stimulation. *Somatosensory Research* 1, 151-168.

MRAOVITCH, S., M. KUMADA and D.J. REIS (1982). Role of the nucleus parabrachialis in cardiovascular regulation in cat. *Brain Research* 232, 57-75.

MUGNAINI, E. and W.H. OERTEL (1985). An atlas of the distribution of GABAergic neurons and terminals in the rat CNS as revealed by GAD immunohistochemistry. In: *Handbook of Chemical Neuroanatomy* volume 4, eds. A. Björklund and T. Hökfelt. Amsterdam: Elsevier, pp. 436-595.

MÜLLER, U.W., K. DEMBOWSKY, J. CZACHURSKI and H. SELLER (1988). Tonic descending inhibition of the spinal cardio-sympathetic reflex in the cat. *Journal of the Autonomic Nervous System* 23, 111-123.

NEIL, J.J. and A.D. LOEWY (1982). Decreases in blood pressure in response to L-glutamate microinjections into the A5 catecholamine cell group. *Brain Research* 241, 271-278.

NOGA, B.R., D.J. KRIELLAARS and L.M. JORDAN (1991). The effect of selective brainstem or spinal cord lesions on treadmill locomotion evoked by stimulation of the mesencephalic or pontomedullary locomotor regions. *Journal of Neuroscience* 11(6), 1691-1700.

PAXINOS, G. and C. WATSON. (1986). *The Rat Brain in Stereotaxic Coordinates.* 2nd edition. Orlando, FL: Academic Press.

PERRI, V., O. SACCHI and C. CASELLA (1970). Electrical properties and synaptic connections of the sympathetic neurons in the rat and guinea-pig superior cervical ganglion. *Pflugers Archives* 314, 40-54.

PILOWSKY, P.M., M.J. WEST and J.P. CHALMERS (1985). Renal sympathetic nerve responses to stimulation, inhibition and destruction of the ventrolateral medulla in the rabbit. *Neuroscience Letters* 60, 51-55.

POLOSA, C., A. MANNARD and W. LASKEY. (1979). Tonic activity of the autonomic nervous system: functions, properties, origins. In: *Integrative Functions of the Autonomic Nervous System*, eds. C.M. Brooks, K. Koizumi and A. Sato. Tokyo: University of Tokyo Press - Elsevier/North Holland, pp. 342-354.

POREE, L.R. and L.P. SCHRAMM (1989). Bilateral injection of muscimol into the rat rostral ventrolateral medulla fails to decrease mean renal nerve activity. *Society for Neuroscience Abstracts* 15, 592.

PURVES, D. and R.I. HUME (1981). The relation of postsynaptic geometry to the number of presynaptic axons that innervate autonomic ganglion cells. *Journal of Neuroscience* 1(5), 441-452.

PURVES, D. and D.J. WIGSTON (1983). Neural units in the superior cervical ganglion of the guinea-pig. *Journal of Physiology* 334, 169-178.

QU, L., R. SHEREBRIN and L.C. WEAVER (1988). Blockade of spinal pathways decreases pre- and postganglionic discharge differentially. *American Journal of Physiology* 255, R946-R951.

RANSON, S.W. and P.R. BILLINGSLEY (1916). Vasomotor reactions from the stimulation of the floor of the fourth ventricle. *American Journal of Physiology* 41, 85-90.

REIS, D.J., S.F. MORRISON and D.A. RUGGIERO (1988). The C1 area of the brainstem in tonic and reflex control of blood pressure. *Hypertension* 11, I8-I13.

ROSS, C.A., D.A. RUGGIERO, T.H. JOH, D.H. PARK and D.J. REIS (1984). Rostral ventrolateral medulla: selective projections to the thoracic autonomic cell column from the region containing C1 adrenaline neurons. *Journal of Comparative Neurology* 228, 168-185.

RUGGIERO, D.A., S.L. CRAVO, V. ARANGO and D.J. REIS (1989). Central control of the circulation by the rostral ventrolateral reticular nucleus: anatomical substrates. *Progress in Brain Research* 81, 49-79.

RUGGIERO, D.A., M.P. MEELEY, M. ANWAR and D.J. REIS (1985). Newly identified GABAergic neurons in regions of the ventrolateral medulla which regulate blood pressure. *Brain Research* 339, 171-177.

SASEK, C.A. and C.J. HELKE (1989). Enkephalin-immunoreactive neuronal projections from the medulla oblongata to the intermediolateral cell column: substance P-immunoreactive neurons. *Journal of Comparative Neurology* 287, 484-494.

SASEK, C.A., M.W. WESSENDORF and C.J. HELKE (1990). Evidence for co-existence of thyrotropin-releasing hormone, substance P and serotonin in ventral medullary neurons that project to the intermediolateral cell column in the rat. *Neuroscience* 35(1), 105-119.

SATO, K., J.-H. ZHANG, T. SAIKA, M. SATO, K. TADA and M. TOHYAMA (1991). Localization of glycine receptor alpha-1 subunit mRNA-containing neurons in the rat brain: an analysis using in situ hybridization histochemistry. *Neuroscience* 43(2/3), 381-395.

SCHRAMM, L.P. and E.S. CHORNOBOY (1982). Sympathetic activity in spontaneously hypertensive rats after spinal transection. *American Journal of Physiology* 243, R506-R511.

SCHRAMM, L.P. and R.H. LIVINGSTONE (1987). Inhibition of renal nerve sympathetic activity by spinal stimulation in rat. *American Journal of Physiology* 252, R514-R525.

SCHRAMM, L.P., A.M. STRACK, K.B. PLATT and A.D. LOEWY (1991). CNS neurons infected by renal injection of pseudorabies virus. *Society for Neuroscience Abstracts* 17, 618.

SEBEL, P.S. and J.D. LOWDON (1989). Propofol: a new intravenous anesthetic. *Anesthesiology* 71, 260-277.

SKOK, V.I. and A.Y. IVANOV (1983). What is the ongoing activity of sympathetic neurons? *Journal of the Autonomic Nervous System* 7, 263-270.

SNEDECOR, G. and W.G. COCHRAN. (1980). *Statistical Methods*. 2nd edition. Ames, IA: The Iowa State University Press.

SPYER, K.M. (1981). Neural organization and control of the baroreceptor reflex. *Review of Physiology, Biochemistry and Pharmacology*. 88, 23-124.

SRIPAIROJTHIKOON, W. and J.M. WYSS (1987). Cells of origin of the sympathetic renal innervation in rat. *American Journal of Physiology* 252, F957-F963.

STANEK, K.A., J.J. NEIL, W.B. SAWYER and A.D. Loewy (1984). Changes in regional blood flow and cardiac output after L-glutamate stimulation of A5 cell group. *American Journal of Physiology* 246, H44-H51.

STEIN, R.D. and L.C. WEAVER (1988). Multi- and single-fibre mesenteric and renal sympathetic responses to chemical stimulation of intestinal receptors in cats. *Journal of Physiology* 396, 155-172.

STEIN, R.D., L.C. WEAVER and C.P. YARDLEY (1989). Ventrolateral medullary neurones: effects on magnitude and rhythm of discharge of mesenteric and renal nerves in cats. *Journal of Physiology* 408, 571-586.

STRACK, A.M., W.B. SAWYER, J.H. HUGHES, K.B. PLATT and A.D. LOEWY (1989b). A general pattern of CNS innervation of the sympathetic outflow demonstrated by transneuronal pseudorabies viral infections. *Brain Research* 491, 156-162.

STRACK, A.M., W.B. SAWYER, K.B. PLATT and A.D. LOEWY (1989a). CNS cell groups regulating the sympathetic outflow to adrenal gland as revealed by transneuronal cell body labelling with pseudorabies virus. *Brain Research* 491, 274-296.

SUN, M.-K. and P.G. GUYENET (1985). GABA-mediated baroreceptor inhibition of reticulospinal neurons. *American Journal of Physiology* 249, R672-R680.

SUN, M.-K., J.T. HACKETT and P.G. GUYENET (1988b). Sympathoexcitatory neurons of rostral ventrolateral medulla exhibit pacemaker properties in the presence of a glutamate-receptor antagonist. *Brain Research* 438, 23-40.

SUN, M.-K., B.S. YOUNG, J.T. HACKETT and P.G. GUYENET (1988a). Reticulospinal pacemaker neurons of the rat rostral ventrolateral medulla with sympathoexcitatory function: an intracellular study in vitro. *Brain Research* 42, 229-239.

SUN, M.-K., B.S. YOUNG, J.T. HACKETT and P.G. GUYENET (1988c). Rostral ventrolateral medullary neurons with intrinsic pacemaker properties are not catecholaminergic. *Brain Research* 451, 345-349.

SVED, A.F., W.W. BLESSING and D.J. REIS (1985). Caudal ventrolateral medulla can alter vasopressin and arterial pressure. *Brain Research Bulletin* 14, 227-232.

TAYLOR, R.F. and L.P. SCHRAMM (1987). Differential effects of spinal transection on sympathetic nerve activities in rats. *American Journal of Physiology* 253, R611-R618.

TAYLOR, R.F. and L.P. SCHRAMM (1988). Spinally mediated inhibition of abdominal and lumbar sympathetic activities. *American Journal of Physiology* 254, R655-R658.

TAYLOR, R.B. and L.C. WEAVER (1992b). Dorsal root input to tonic firing of renal and mesenteric sympathetic nerves in rats. *American Journal of Physiology,* (in press).

TAYLOR, R.B. and L.C. WEAVER (1992a). Spinal stimulation to locate preganglionic neurons controlling the kidney, spleen or intestine. *American Journal of Physiology* 263, H1026-H1033.

TER HORST, G.J., J.C.V.M. COPRAY, R.S.B. LIEM and J.D. VAN WILLIGEN (1991). Projections from the rostral parvocellular reticular formation to pontine and medullary nuclei in the rat: involvement in autonomic regulation and orofacial motor control. *Neuroscience* 40(3), 735-758.

VAN BOCKSTAELE, E.J., V.A. PIERIBONE and G. ASTON-JONES (1989). Diverse afferents converge on the nucleus paragigantocellularis in the rat ventrolateral medulla: retrograde and anterograde tracing studies. *Journal of Comparative Neurology* 290, 561-584.

VARNER, K.J., C.L. GROSSKREUTZ, B.F. COX and M.J. BRODY (1989). Differential regulation of sympathetic nerve activity by lateral and medial subregions of the rostral ventral medulla. *Progress in Brain Research* 81, 99-103.

VASQUEZ, E.C., S.J. LEWIS, K.J. VARNER and M.J. BRODY (1992). Chronic lesion of rostral ventrolateral medulla in spontaneously hypertensive rats. *Hypertension* 19(suppl II), 154-158.

WALLIS, D.I. and R.A. NORTH (1978). Synaptic input to cells of the rabbit superior cervical ganglion. *Pflugers Archives* 374, 145-152.

WANG, S.C. and S.W. RANSON (1939). Autonomic responses to electrical stimulation of the lower brain stem. *Journal of Comparative Neurology* 71(3), 437-455.

WARD, D.G. (1988). Stimulation of the parabrachial nuclei with monosodium glutamate increases arterial pressure. *Brain Research* 462, 383-390.

WERMAN, R., A. DAVIDOFF and M.H. APRISON (1968). Inhibitory action of glycine on spinal neurons in the cat. *Journal of Neurophysiology* 31, 81-95.

WEST, M.J., W.W. BLESSING and J.P. CHALMERS (1981). Arterial baroreceptor reflex function in the conscious rabbit after brainstem lesions coinciding with the A1 group of catecholamine neurons. *Circulation Research* 49, 959-970.

WESTLUND, K.N., R.M. BOWKER, M.G. ZIEGLER and J.D. COULTER (1983). Noradrenergic projections to the spinal cord of the rat. *Brain Research* 263, 15-31.

WILLETTE, R.N., P.P. BARCAS, A.J. KRIEGER and H.N. SAPRU (1983b). Vasopressor and depressor areas in the rat medulla: identification by microinjection of L-glutamate. *Neuropharmacology* 22(9), 1071-1079.

WILLETTE, R.N., A.J. KRIEGER, P.P. BARCAS and H.N. SAPRU (1983a). Medullary gamma-amniobutyric acid (GABA) receptors and the regulation of blood pressure in the rat. *Journal of Pharmacology and Experimental Therapeutics* 226, 893-899.

WILLETTE, R.N., S. PUNNEN, A.J. KRIEGER and H.N. SAPRU (1984b). Interdependence of rostral and caudal ventrolateral medullary areas in the control of blood pressure. *Brain Researc'* 321, 169-174.

WILLETTE, R.N., S. PUNNEN, A.J. KRIEGER and H.N. SAPRU (1984a). Hypertensive response following stimulation of opiate receptors in the caudal ventrolateral medulla. *Neuropharmacology* 23(4), 401-406.

WILLETTE, R.N., S. PUNNEN-GRANDY, A.J. KRIEGER and H.N. SAPRU (1987). Differential regulation of regional vascular resistance by the rostral and caudal ventrolateral medulla in the rat. *Journal of the Autonomic Nervous System* 18, 143-151.

YARDLEY, C.P., J.M. ANDRADE and L.C. WEAVER (1989b). Evaluation of cardiovascular control by neurons in the dorsal medulla of rats. *Journal of the Autonomic Nervous System* 29, 1-12.

YARDLEY, C.P., R.D. STEIN and L.C. WEAVER (1989a). Tonic influences from the rostral medulla affect sympathetic nerves differentially. *American Journal of Physiology* 256, R323-R331.

YIP, J.W. (1986). Migratory patterns of sympathetic ganglioblasts and other neural crest derivatives in chick embryos. *Journal of Neuroscience* 6(12), 3465-3473.

YOUNG, A.B. and S.H. SNYDER (1973). Strychnine binding associated with glycine receptors of the central nervous system. *Proceedings of the National Academy of Science USA* 70, 2832-2836.

ZEIGLER, M.T. (1988). Anatomical relationship between the lower thoracolumbar sympathetic chain and the spinal cord of the rat. Master of Arts Thesis, John Hopkins University, Baltimore, Maryland, pp.1-48.