INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

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

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

U·M·I

University Microfilms International
A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
313.761-4700 800.521-0600

Order Number 9137022

Ventral medullary organization for cardio-respiratory control

Jung, Ranu, Ph.D.

Case Western Reserve University, 1991

U·M·I

300 N. Zeeb Rd.
Ann Arbor, MI 48106

VENTRAL MEDULLARY ORGANIZATION
FOR CARDIO-RESPIRATORY CONTROL

by

RANU JUNG

Submitted in partial fulfillment of the requirements
for the Degree of Doctor of Philosophy

Thesis Advisor: Peter G. Katona, Sc.D.

Department of Biomedical Engineering
CASE WESTERN RESERVE UNIVERSITY

January 1991

CASE WESTERN RESERVE UNIVERSITY

GRADUATE STUDIES

We hereby approve the thesis of

RANU JUNG

candidate for the Ph. D.

degree.*

Signed:

B. S. Watson
(Chairman)

Matthew N. Long

Eugene M. Brown

John M. Miko

Walter C. Cresswell

E. Merrill Adams

Date Dec 16, 90

*We also certify that written approval has
been obtained for any proprietary material
contained therein.

I grant to Case Western Reserve University the right to use this work, irrespective of any copyright, for the University's own purposes without cost to the University or to its students, agents and employees. I further agree that the University may reproduce and provide single copies of the work, in any format other than in or from microforms, to the public for the cost of reproduction.

VENTRAL MEDULLARY ORGANIZATION FOR CARDIO-RESPIRATORY CONTROL

Abstract

by

RANU JUNG

Arterial baroreceptor stimulation inhibits both arterial pressure (baro-pressure reflex) and ventilation (baro-ventilatory reflex). Regions of the ventral medulla, both near the medullary surface (VMS) and deeper in the rostral and caudal ventrolateral medulla (RVLM and CVLM respectively), are involved not only in vasomotor control and the baro-pressure reflex, but also in respiratory control. The aim of this research was to determine if these regions are also part of the pathway for the baro-ventilatory reflex.

RVLM neural structures were blocked in anesthetized dogs by unilateral focal cooling using a needle probe. The results affirmed that RVLM neural structures of the dog affect control of arterial pressure and ventilation and play a role in the baro-pressure reflex. The RVLM structures were also found to influence the baro-ventilatory reflex. Absence of detailed information regarding neural structures within the dog medulla, and technical difficulties of using the ventral approach to the medulla prompted the use of rats for further studies.

The role of neural structures adjacent to the VMS of anesthetized rats were evaluated by surface applications of various drugs (pentobarbital, nicotine, bicuculline). The results confirmed that these structures affect arterial pressure and the baro-pressure reflex, at least in part, through GABA_A receptors. These structures also affect ventilation though not via GABA_A receptors. Additionally, they do not play a significant role in the baro-ventilatory reflex.

Neural structures in the CVLM of anesthetized rats were blocked by microinjections of excitatory amino acid antagonists. Unilateral microinjections of kynurenic acid, a non-specific glutamatergic antagonist, caused respiratory arrest, and raised the baseline blood pressure. Bilateral block abolished the baro-pressure reflex. Because of the respiratory arrest, the baro-ventilatory reflex could not be determined. Further experiments indicated that ventilation is sustained by both NMDA and non-NMDA receptor activation, while the increase in baseline arterial pressure is only mediated via NMDA receptors. The results confirmed that the CVLM forms an obligatory synapse for mediating the baro-pressure reflex via both NMDA and non-NMDA receptors.

The effects of neural blockade on ventilation was examined with a hypothetical neural network model. This model was then used to suggest experiments to further elucidate the role of ventrolateral medullary structures in cardio-respiratory control.

**We dance round in a ring and suppose,
But the Secret sits in the middle and knows.**

-Robert Frost
The Secret Sits

Dedicated
To
My parents Sarla and Padam and my husband Jimmy

ACKNOWLEDGMENTS

I express my sincere gratitude to Dr. Peter Katona whose guidance, patience, and effort have supported me during the course of my Ph.D. From near and from afar he has made extraordinary efforts in guiding me. I am thankful to my committee members Drs. Merrill Adams, Eugene Bruce, Neil Cherniack, Matthew Levy, and Jyoti Mitra for their suggestions, guidance, time, and effort. I am specially thankful to Dr. Eugene Bruce for being my advisor while Dr. Katona was on sabbatical in Germany. Since then he has been 'around the corner' and has continued to support me.

I thank Dr. Merrill Adams for use of his laboratory for the dog studies and Dr. Eugene Bruce for use of his laboratory for some of the rat studies. I also thank Drs. N.B. Dev and Jyoti Mitra for kindly letting me use their laboratory for the brain sectioning in spite of the inconvenience. Thanks also to John Butts for the data collection program that was wonderfully 'tailored' to my needs. I also thank Dr. David Averiel, Dr. Katherine Merritt, Paritosh Dhawale, Scott Heubner, Mohammed Khatib, Mike Sammon, and Rupa Trivedi for their help in several small and big ways during the course of my Ph.D.

William Timmons, my present office mate and Dr. Roberto Maass, my past lab partner, and Ditte Wolin I sincerely appreciate your friendship and support. Thanks also to Doris Fink, Mary Jo DeSario, and Donna Waldron for their secretarial help. Donna specially has been tremendously helpful in 'faxing' papers to Dr. Katona

in Washington. To Marc D. Thames, M.D. I am thankful for letting me accomodate my time in his laboratory for the past few months thus allowing me to complete my Ph.D.

Finally, my dear husband Jimmy has been extremely encouraging, patient, and helpful. My parents and mother in-law have been very supportive. I feel lucky to have their love and blessings.

TABLE OF CONTENTS

ABSTRACT.....	ii
DEDICATION.....	v
ACKNOWLEDGMENTS.....	vi
LIST OF FIGURES.....	xi
LIST OF TABLES.....	xiv
LIST OF ABBREVIATIONS.....	xv
I. INTRODUCTION	
Objectives.....	1
Organization of the dissertation.....	3
II. REVIEW and RATIONALE	
Literature review.....	5
Rationale for experiments.....	16
References.....	20
III. EFFECTS ON BAROREFLEX CONTROL OF ARTERIAL PRESSURE AND VENTILATION OF FOCAL COOLING IN THE ROSTRAL MEDULLA OF THE DOG	
Introduction.....	28
Methods.....	29
Results.....	33

	Discussion.....	36
	References.....	40
IV.	TONIC AND BAROREFLEX EFFECTS ON ARTERIAL PRESSURE AND VENTILATION OF PENTOBARBITAL AND NICOTINE ON THE RAT VENTRAL MEDULLARY SURFACE	
	Introduction.....	48
	Methods.....	48
	Results.....	51
	Discussion.....	53
	References.....	56
V.	ROLE OF VENTRAL MEDULLARY SURFACE GABA _A RECEPTORS IN MEDIATING BAROREFLEX INHIBITION OF ARTERIAL PRESSURE AND VENTILATION IN THE RAT	
	Introduction.....	60
	Methods.....	61
	Results.....	63
	Discussion.....	65
	References.....	68
VI.	CARDIO-RESPIRATORY RESPONSES TO GLUTAMATERGIC ANTAGONISTS IN THE CAUDAL VENTROLATERAL MEDULLA OF RATS	

Introduction.....	75
Methods.....	76
Results.....	82
Discussion.....	88
References.....	96
VII. CONCLUSIONS	
Overview of results.....	111
Discussion.....	113
VIII. FUTURE WORK	
Neural network model for inspiratory pattern generation.....	124
Suggestions for future research.....	132
References.....	138
BIBLIOGRAPHY.....	150

LIST OF FIGURES

Fig. II.1	Schematic representation for baro-pressure reflex pathways....	27
Fig. III.1	Schematic cross sections of the dog brain-stem indicating sites at which focal cold block was achieved.....	43
Fig. III.2	Systemic arterial pressure (SAP) and diaphragmatic activity (EMG), before (left panel) and approximately 3 minutes after cooling probe tip to 16°C (right panel).....	44
Fig. III.3	Average baroreflex responses before and after focal cold block.....	45
Fig. III.4	Regression fits to the baro-pressure response curves.....	46
Fig. III.5	Regression fits to the baro-ventilatory response curves.....	47
Fig. IV.1	Effects of ADN stimulation.....	57
Fig. IV.2	Effects of pentobarbital application to the VMS.....	58
Fig. IV.3	Effects of nicotine application to the VMS.....	59
Fig. V.1	Effects of ADN stimulation.....	71
Fig. V.2	Effects of BIC application to the VMS.....	72
Fig. V.3	Maximum responses to ADN stimulation (absolute change).....	73
Fig. V.4	Maximum responses to ADN stimulation (percentage change).....	74
Fig. VI.1	Typical Systemic arterial pressure (SAP), respiratory flow (FLOW) and tidal volume (VT) responses to unilateral KYN (5 nmol in 50 nl) microinjection in the VLDA.....	101
Fig. VI.2	Time profiles for 4 rats of mean arterial pressure (MAP), ventilation (VE), respiratory frequency (F), and tidal	

	volume (VT) after unilateral microinjection of 5 nmol KYN in the CAUDAL sites.....	102
Fig. VI.3	Baro-pressure response to supramaximal aortic depressor nerve stimulation before (left) and after (right) bilateral microinjection of KYN (5 nmol) in the VLDA.....	103
Fig. VI.4	Typical SAP, FLOW, and VT responses to unilateral KYN (1.58 nmol in 31 nl) microinjection in the VLDA.....	104
Fig. VI.5	Time profiles for 4 rats of MAP, $\dot{V}E$, F, and VT after unilateral microinjection of 1.58 nmol KYN in the ROSTRAL (left panel) and CAUDAL (right panel) sites.....	105
Fig. VI.6	Typical SAP, FLOW, and VT responses to unilateral 2APV (2.7 nmol) microinjection in the CVLM.....	106
Fig. VI.7	Time profiles for 5 rats of MAP, $\dot{V}E$, F, and VT after unilateral microinjection of 2.7 nmol 2APV in the ROSTRAL (left panel) and CAUDAL (right panel) sites.....	107
Fig. VI.8	Typical SAP, FLOW, and VT responses to unilateral CNQX (0.257 nmol) microinjection in the CVLM.....	108
Fig. VI.9	Time profiles for 5 rats of MAP, $\dot{V}E$, F, and VT after unilateral microinjection of 0.257 nmol CNQX in the ROSTRAL (left panel) and CAUDAL (right panel) sites.....	109
Fig. VI.10	Coronal sections indicating ROSTRAL (left panel), and CAUDAL (right panel) ventrolateral medullary sites where KYN, 2APV, and CNQX were microinjected.....	110
Fig. VII.1	Schematic representation of neural structures under the ventral medullary surface (VMS).....	121
Fig. VII.2	Schematic representation for baro-reflex pathways.....	122
Fig. VII.3	Schematic representation for baseline and baroreflex control of arterial pressure.....	123

Fig. VIII.1	Neural network circuit for the respiratory pattern generating model.....	142
Fig. VIII.2	Schematic electrical equivalent for a neuron.....	143
Fig. VIII.3	Normal neural activity of all the respiratory pools shown in Fig. 1.....	144
Fig. VIII.4	Effect of blocking synaptic input from left EI neurons to right I neurons.....	145
Fig. VIII.5	Effect of blocking synaptic input from left I neurons to right I neurons.....	146
Fig. VIII.6	Effect of blocking synaptic input from right I neurons to right LI neurons.....	147
Fig. VIII.7	Effect of blocking synaptic input from left EI neurons to right EI and I neurons, and blocking synaptic input from left I neurons to right I neurons.....	148
Fig. VIII.8	Effect of block of synaptic input from the left EI neurons to the right I neurons, from the right EI neurons to the left I neurons, and the reciprocal inputs between the left and right I neurons.....	149

LIST OF TABLES

Table IV.1	Percentage change in baseline values of mean arterial pressure (MAP), ventilation (V_n), respiratory frequency (F), peak diaphragmatic activity (Edi) in 6 dogs on unilateral focal cooling.....	42
Table IV.2	Percentage change in MAP and V_n (Δ MAP and ΔV_n repectively) between threshold and saturation on baroreceptor stimulation before and after unilateral focal cold block in 6 dogs.....	42
Table V.1	Effects of bicuculline (BIC) application to the ventral medullary surface of 7 rats on baseline mean arterial pressure (MAP), ventilation ($\dot{V}_E$), respiratory frequency (F), and tidal volume (V_T).....	70
Table V.2	Effects of bicuculline (BIC) application to the ventral medullary surface of 7 rats on baroreflex responses (Δ MAP, $\Delta \dot{V}_E$, ΔF , and ΔV_T).....	70
Table VI.1	Baseline mean arterial pressure (MAP) and change in MAP on aortic depressor nerve stimulation (BPR) before and after bilateral microinjection of kynurenic acid (KYN), 2-amino-5-phosphonovaleric acid (2APV), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in the caudal depressor area.....	100
Table VII.1	Qualitative summary of the principal results from different experiments.....	120
Table VIII.1	Parameter values for each of the neurons.....	140
Table VIII.2	Pacemaker properties for the left and right pacemakers.....	140
Table VIII.3	Synaptic weights for different connections.....	141

LIST OF ABBREVIATIONS

2APV	2-Amino-5-phosphonovaleric acid (a competitive NMDA receptor antagonist)
ADN	Aortic Depressor Nerve
ANOVA	Analysis of Variance
BIC	Bicuculline (a GABA _A receptor antagonist)
BPR	Baro-pressure reflex
C ₄	4th cervical nerve root
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione (a competitive non-NMDA receptor antagonist acting on AMPA, Kainate and Quisqualate receptors)
CSF	Cerebro Spinal Fluid
CSP	Carotid Sinus Pressure
CVLM	Caudal Ventrolateral Medulla
DC	Dorsal Cochlear nucleus
DRG	Dorsal Respiratory Group
E	Expiratory bulbospinal neurons
EAA	Excitatory amino-acid
Edi	Peak integrated diaphragmatic activity (in arbitrary units)
EI	Early Inspiratory propriobulbar neurons
EMG	Electromyogram
F	Frequency (in breaths/min)
GABA	γ-amino-butyric Acid (an inhibitory amino-acid)
G _{MAP}	baro-pressure gain
G _{Va}	baro-ventilatory gain
I	Inspiratory bulbospinal neurons
KYN	Kynurenic acid (a broad-band excitatory amino acid receptor antagonist)
LI	Late Inspiratory propriobulbar neurons
MAP	Mean Arterial Pressure
NMDA	N-methyl-D-aspartate (an excitatory amino acid)
NPA	Nucleus Para Ambiguus
NRA	Nucleus Retro Ambiguus
NTS	Nucleus Tractus Solitarius
rVRG	rostral Ventral Respiratory Group
RVLM	Rostral Ventrolateral Medulla
SAP	Systemic Arterial Pressure
TBS	Tris Buffered Saline
VE	Ventilation (in ml/min)
VLPA	Ventrolateral Pressor Area

VLDA	Ventrolateral Depressor Area
VLM	Ventrolateral Medulla
VMS	Ventral Medullary Surface
Vn	Ventilation (in arbitrary units)
VRG	Ventral Respiratory Group
VT	Tidal Volume (in ml)

CHAPTER I

INTRODUCTION

OVERVIEW AND OBJECTIVES

It is well known that the cardiovascular and respiratory systems interact as evidenced by their coordinated responses to hypercapnia, hypoxia and exercise. However, the neurophysiological and neuropharmacological mechanisms as well as anatomical substrates and pathways involved in the central interaction of these two systems remain undetermined. While it is likely that certain major areas of the brainstem subserve strictly cardiovascular or respiratory functions, there is increasing evidence that some areas may be involved in the control of both systems. An understanding of the central organization for the coupling of the two systems would not only increase the knowledge of central mechanisms of cardio-respiratory control but also help understand heretofore vaguely understood phenomena like exercise hyperpnea, hemorrhagic hyperpnea and cardio-respiratory responses to central hypoxia.

Within the ventrolateral medulla and near the ventral medullary surface lie several neural structures that control arterial pressure and ventilation. These neural structures are also part of the medullary pathways for the baroreflex inhibition of arterial pressure (baro-pressure reflex). However, the pathways for the less well understood baroreflex inhibition of ventilation (baro-ventilatory reflex) are unknown. It has been shown that endogenous inhibitory amino-acids like GABA and excitatory

amino-acids like L-glutamate affect vasomotor tone and ventilation, as well as act on receptors at some of the synaptic connections in the baro-pressure pathway. Just as the anatomical substrate for the baro-ventilatory reflex is unknown, the neurotransmitters and the receptors that mediate the synaptic connections also remain to be determined.

This thesis is based on two fundamental concepts: (1) Responses to arterial baroreceptor stimulation can be used as a tool to investigate the central organization of the integrated cardio-respiratory control since such stimulation affects both the cardiovascular and respiratory systems. (2) The same neural structures that affect steady state control of arterial pressure, ventilation, and both baroreflexes are likely sites for mediating interactions between the cardiovascular and respiratory systems. Therefore, the goal of this research was to determine the role of these neural structures in the baroreflex control of both arterial pressure and ventilation. The specific hypotheses tested were:

- (1) Neural structures near the ventral medullary surface (VMS) and deeper in the rostral and caudal ventrolateral medulla (RVLM and CVLM respectively) that affect steady state vasomotor tone and ventilation are also part of the baro-pressure reflex and the baro-ventilatory reflex pathways, and
- (2) The baroreflexes are mediated by GABA_A receptors for the endogenous inhibitory amino-acid GABA, and *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors for excitatory amino-acids like L-glutamate.

If hypothesis 1 is true it would suggest that neural structures near the VMS, and in the rostral and caudal ventrolateral medulla are important areas for cardio-respiratory interaction and control. Similarly, if hypothesis 2 is true then our understanding of the of the neurotransmitters mediating cardio-respiratory control would be improved. This information has important implications for understanding central mechanisms governing normal as well as pathological states of control and interaction between the cardiovascular and respiratory systems.

ORGANIZATION OF THE DISSERTATION

Chapter I presents a brief introduction, specifies the objectives, and outlines the organization of the dissertation. Chapter II contains a brief literature survey and gives the rationale for the different experimental series performed. The role of the RVLM neural structures in the baro-pressure and baro-ventilatory reflexes in the dog was determined in the study presented in Chapter III. As explained in Chapter II under "Rationale for experiments", the experimental subject had to be changed from the dog to the rat. Chapters IV and V present the results used to test the hypothesis that neural structures near the VMS of the rat are part of the baro-pressure and baro-ventilatory pathways. Chapter V also presents the results from experiments done to determine whether GABAergic receptors on neural structures close to the VMS mediate the baro-pressure and baro-ventilatory reflexes. The role of the CVLM neural structures in the baroreflexes in the rat was examined in experiments presented in

Chapter VI. This chapter also presents the effects on baroreflexes of excitatory amino-acids acting on neural structures in the CVLM. Chapter VII presents the overall conclusion of the research. Finally, Chapter VIII presents possibilities for future work. It discusses a neural network model for generating respiratory patterns, suggests future research avenues motivated by this neural network and the results obtained in the experiments performed.

Each chapter is self-contained, with a brief introduction and relevant review of literature for the specific hypothesis tested in the chapter. The experimental methodologies are described, and the results presented. The chapters are concluded with a discussion of the results and a list of pertinent references.

CHAPTER II

REVIEW AND RATIONALE

LITERATURE REVIEW

The neural pathways and the role of the brainstem in cardiovascular and respiratory control have been investigated by several methods. These methods have divided the brainstem into regions based on anatomical evidence, functional significance, and responses to electrophysiological and chemical stimuli. The following is a brief description of the pathways and neurotransmitters that may be part of the arterial and respiratory baroreflexes and a short discussion of respiratory rhythm and pattern generation.

Neural pathways for Baroreflex control of Arterial Pressure

Arterial baroreceptor stimulation causes a reflex decrease in blood pressure (baro-pressure reflex) [10,32,40]. Figure 1 represent a schematic outline for the major central medullary pathway for mediating this reflex. The figure is adapted from Figure 8 in [59] and based on the following literature review.

Nucleus tractus solitarius

Baroreceptor afferents from the carotid sinuses and the aortic arch have their first synapse in the nucleus tractus solitarius (NTS) in the dorsal medulla [8,37]. In

the rat and the cat, they have been found to terminate with both ipsilateral and contralateral projections most often rostral to the obex [15,29]. Gamma aminobutyric acid (GABA), opioids, alpha-adrenergic agonists, atrial natriuretic factor, and Substance-P have all been found to act at the NTS to modulate the baroreceptor reflex control of blood pressure [35,43,46,49]

Ventral medulla

Several investigators have tried to determine areas in the ventral medulla that are important in maintaining the vasomotor tone and/or are involved in the baroreflex control of blood pressure. Though the first synapse for the baroreceptor afferents has been identified in the nucleus tractus solitarius (NTS), the entire baroreflex arc is still under investigation. Neurons both in the rostral ventrolateral medulla (RVLM) near the nucleus facialis and the region of the caudal ventrolateral medulla (CVLM) located between the nucleus ambiguus and lateral reticular nucleus have been implicated in vasomotor control and the arterial pressure baroreflex [2,3,5,8,12,54,60,61,63].

The RVLM receives projections from the NTS and the CVLM, and is considered to be the final point of sympathetic discharge outflow to the sympathetic preganglionic neurons [2,8,12,55,63]. This concept is supported by extracellular recordings from neurons in this region that indicate that their activity is suppressed by arterial baroreceptor stimulation [3,9]. The RVLM is also considered a pressor

area since stimulation of this area by excitatory substances such as kainic acid and l-glutamic acid cause an increase in systemic arterial pressure (SAP) [8,12]. It has been suggested that the C1 adrenergic neurons in this region are inhibited by GABAergic inputs [28,62,63] and mediate the sympatho-inhibitory baroreflex [54].

Considerable evidence suggests that the CVLM forms an obligatory synapse in mediating the arterial baroreflex [2,3,25,28,35,60,61]. The CVLM has direct glutamatergic projections from the NTS and reciprocal GABAergic connections with the RVLM [2,3,60,61,63]. The CVLM is also considered to be a vasodepressor area since stimulation by substances such as L-glutamate cause a decrease in SAP [6,12,27,62]. Glutamatergic projections from the NTS to the CVLM play an important role in the baroreflex control of SAP [2,3,25,34,60].

Other regions of the brain with medullary projections

Besides the NTS and the ventral medulla, several different pathways between these regions and the pons, forebrain, and hypothalamus can influence circulation and the baroreflex control of SAP via several putative neurotransmitters [8,31,39]. The hypothalamus, in particular, plays an important role in the neurohumoral response to baroreceptor stimulation [8,12,23]. Some of the pertinent reviews discussing the role of non-medullary regions in the cardiovascular control are listed as references [8,12,13,23,39].

Neural pathways for Baroreflex control of Ventilation

Besides inhibiting blood pressure, arterial baroreceptor stimulation also inhibits ventilation (baro-ventilatory reflex) [10,32,40,58], mainly by inhibiting respiratory frequency. The effects on tidal volume appear to be species dependent; on arterial baroreceptor stimulation a reflex inhibition of tidal volume occurs in the cat and the rat [40,58], while tidal volume increases in the dog [10,32]. The central organization for the baroreflex control of ventilation, however, has been studied only very sparsely. Using intracellular recordings in the ventral medulla of anesthetized cats, Richter and Seller have suggested direct inhibitory connections from baroreceptor afferents to inspiratory neurons [53] since these neurons were hyperpolarized by increasing either intracarotid pressure or aortic nerve stimulation. There are several medullary pathways that could result in the baro-ventilatory reflex. Before discussing the potential pathways for the baro-ventilatory reflex, a brief review of the medullary organization of respiratory neurons for conventional respiratory pattern generation and control is presented since these neural structures may be a part in the baro-ventilatory reflex pathway.

Medullary "respiratory neurons"

Several medullary neural populations have been that have neurons that fire with some relation to the respiratory activity. These neurons are classified depending on when they fire in the respiratory cycle. If anatomical and functional projections

exist from these neurons to the spinal cord they are considered bulbo-spinal; if they have projections to other medullary regions they are considered propriobulbar neurons. The following listing is a synopsis of the major medullary neuronal pools based on anatomical and functional connections and electrophysiological activities obtained from [17,18,19,20,38,48,49,52]. All neural pools are symmetrical between the two medullary halves [20]. The following abbreviations are used:

I: Inspiratory activity related neurons.

E: Expiratory activity related neurons

NPA: Nucleus paraambiguus

NRA: Nucleus retroambiguus

NTS: Nucleus tractus solitarius

Dorsal Respiratory Group (DRG)

This group is predominantly composed of inspiratory (I) bulbospinal neurons in the dorsal medulla in the ventrolateral part of the NTS. The DRG neurons in the rat have been found to be very sparse, so the following classification was obtained from studies in the cat. The main neuron types are:

- (1) R_{α} neurons - inflation of lungs terminates their discharge and they cannot be re-excited by inflation during expiration.
- (2) R_{β} neurons - inflation of lungs increases and then terminates their discharge; they can be re-excited by inflation during expiration.

(3) P cells - their discharge pattern follows changes in lung volumes; they are not affected significantly by central inspiration related activity.

Ventral Respiratory Group (VRG)

This group consists of neurons located in the ventrolateral medulla.

(1) Bötzinger neurons

They are the most rostral group in the VRG. They are predominantly expiratory (E) bulbospinal neurons near the nucleus retrofacialis. They have an augmenting pattern of discharge which peaks late in expiration. They project to 1) the contralateral NTS, 2) the ipsilateral NRA, 3) the phrenic motoneurons in the spinal cord, and 4) the ipsilateral bulbospinal I neurons of NPA.

(2) Nucleus Paraambiguus neurons (NPA)

These neurons lie in the ventrolateral medulla in the region caudal to the Bötzinger complex, and extend into the medulla slightly caudal to the obex. This region also appears to correspond to the rostral ventral respiratory group described by Ellenberger et. al [18]. The neurons are predominantly I neurons, though in the caudal part of NPA they are intermingled with E neurons. Several groups of I neurons have been shown to exist, and are briefly described below.

(a) Bulbospinal I neurons

These are inspiratory pre-motor neurons of the NPA, inhibited by lung stretch receptors and excited by pulmonary stretch receptors. Their discharge is increased by hypercapnia. They have an augmenting pattern of activity with a peak in late inspiration. They receive projections from 1) the ipsilateral NTS (less from contralateral NTS), 2) the contralateral early-burst I neurons, and 3) the Bötzing complex (bilaterally). They, in turn, project to 1) the contralateral (predominant) and ipsilateral phrenic motoneurons in the spinal cord, 2) the ipsilateral bulbospinal I neurons of NPA, 3) the contralateral NPA bulbospinal I neurons and 4) the contralateral NRA bulbospinal E neurons.

(b) EI neurons

These are early burst propriobulbar I neurons in the rostral part of NPA. They fire immediately after expiration with peak activity early in inspiration which terminates in mid to late inspiration. They are inhibited by lung stretch receptors.

They receive projections from 1) the DRG I neurons (mainly ipsilateral, anatomical evidence), 2) the Bötzing E neurons (bilaterally, anatomical evidence), 3) the bulbospinal I neurons of NPA (anatomical evidence), and 4) the contralateral EI neurons. They project to 1) the contralateral EI neurons, 2) predominantly contralateral E neurons of NRA, and 3) the contralateral bulbospinal I neurons of NPA.

(c) LI neurons

These are late inspiratory propriobulbar interneurons having an augmented pattern of discharge during the late inspiratory phase with peak activity at end of inspiration. They receive projections from 1) the early burst I neurons of NPA (electrophysiological evidence) and 2) the bulbospinal I neurons of NPA (speculative). They project to bulbospinal I neurons of NPA and NTS (indirect evidence).

(d) PI neurons

These are post-inspiratory propriobulbar interneurons of NPA located rostral to obex, between NRA and NTS. They are active in the post-inspiratory phase showing an early peak firing with declining activity. They are inhibited strongly in the inspiratory phase and they disinhibit E neurons of NRA post inspiration. These neurons are excited by inputs from the pons, peripheral chemoreceptor and larynx stimulation. They receive projections from 1) the pons, 2) the bulbospinal I neurons and 3) the early burst I neurons (indirect evidence). They project to the ipsilateral bulbospinal I neurons of NPA (indirect evidence). They also gate the activity of E neurons of NRA (indirect evidence).

(3) Nucleus Retroambiguus neurons (NRA)

These are expiration (E) related bulbo-spinal neurons located caudal to the NPA and span the ventrolateral medulla caudal to the obex till the pyramidal

decussation. This region also corresponds to the caudal ventral respiratory group described by Ellenberger et al. in the rat [17,18].

They have an augmenting discharge pattern with peak in late expiration and receive projections from 1) the ipsilateral and contralateral I neurons of NTS and 2) the contralateral early burst I neurons. They project to expiratory internal intercostal neurons in the thoracic spinal cord (primarily contralateral projections in the cat; approximately equal bilateral projection in the rat).

Respiratory rhythm and pattern generation

Lesion studies in the cat, rat, and the in vitro brainstem-spinal cord preparation of the neonate rat, have suggested that neural structures for respiratory rhythm and pattern generation lie in the medulla [4,21,22,30,50]; though for normal respiration other neuronal structures such as those in the pons may be necessary [30]. The mechanism for respiratory rhythm and pattern generation, however, remains equivocal. One school of thought proposes that "neural oscillators" provide both respiratory rhythm and pattern generation, i.e. a population of neurons with respiratory- related activity are interconnected in a neural network that oscillates to provide the timing as well as the drive to respiratory motoneurons [20]. Another school of thought proposes that the neural structures providing respiratory timing are distinct from those that generate the respiratory pattern, the former being provided by some "pacemaker" mechanism [21,22,50].

Respiratory pattern generation by the neural oscillator mechanism has often been explained by the three phase theory of Richter et al. [52]. This theory proposes that interconnections between neurons of the VRG (described earlier) are capable of generating a respiratory rhythm as well as the pattern [20,52] such that for a typical breath there is an inspiratory phase, a postinspiratory phase (separated by a brief pause from the inspiratory phase), and an expiratory phase. A mathematical neural network model based on this theory has recently been presented [7]. It also has been suggested that there is no singular oscillator for central respiratory rhythm generation, rather interactions between neural oscillators distributed over several medullary sites provide the basis for respiratory rhythmogenesis [30].

The "pacemaker" neuron proposal provides the respiratory timing input to other respiratory-related neurons that, in turn, provide the pattern of respiration (i.e. shape of the respiratory burst) [4,21,22,50]. The neurons forming the burst pattern include I and E pre-motoneurons and motoneurons, as well as those affecting the respiratory airways [21,22]. Neural circuitry coordinates the activity of the I and E neurons resulting in the shape of each breath. External inputs, such as vagal feedback, can affect these burst-forming neurons. The rhythm generation is caused by "pacemaker neurons" that are, however, "conditionally bursting" neurons [21,22], in that they require extrinsic excitatory input for rhythmic oscillation. They can also be inhibited by extrinsic inputs and/or their rhythm is modulated by inputs that can affect their membrane conductances. Studies in the neonatal rat brainstem-spinal cord

preparation, suggest that pre-inspiratory neurons near the Bötzing complex in the RVLM are the pacemaker neurons [4,21,22]. The rhythmic activity of these neurons is not affected by lesions in other caudal regions of the ventrolateral medulla that disrupt the recorded C_4 activity (indicative of inspiratory activity) or after complete transection of the caudal medulla from the rostral medulla [4,50]. These lesion sites in the caudal ventrolateral medulla may be the regions for the inspiratory pattern generator [4,47].

Potential pathways for the baro-ventilatory reflex

Baroreceptor stimulation may inhibit ventilation via several pathways. The reflex could occur because of direct inhibition of the dorsal respiratory group (DRG) by neurons in the NTS (the site of first synapse for the baroreceptor afferent input). Alternatively, the respiratory neurons in the ventral medulla, the ventral respiratory group (VRG), could be directly inhibited by neural projections from the NTS.

The neurons of the ventral respiratory group lie adjacent to those known to affect blood pressure control [17,18,19,45]. As shown by stimulation, lesioning, or cooling of neural structures both adjacent to the ventral medullary surface (VMS) or deeper in the ventrolateral medulla, areas that affect arterial pressure overlap areas which, when disturbed, alter respiration [1,11,14,24,36,41,42,44,45,63]. Similarly, microinjections of excitatory amino-acids into specific regions of the ventral medulla can elicit both blood pressure and respiratory responses [6,24,36]. Hence, there may

exist direct connections between the neural structures that affect blood pressure and those that affect ventilation. Besides the possible direct effects on respiratory neurons from the baroreceptor afferents in the NTS, the baro-ventilatory reflex could be elicited via projections from ventral medullary neural structures that are part of the baro-pressure pathway to the ventral respiratory group. The baro-pressure pathway consists of projections from the NTS to the caudal ventrolateral depressor area (VLDA) to the rostral ventrolateral pressor area (VLPA). It has been suggested that, in the rat, neurons in the ventrolateral depressor area may not directly influence the respiratory baroreflex [60] even though they are thought to be an obligatory part of the pathway for baroreflex control of blood pressure.

Central regulation of respiratory neurons can involve several putative neurotransmitters which may be monoamines, neuropeptides or amino-acids [16,47]. Since both l-glutamate and GABA affect central respiratory activity [6,36,47,59,63] and have an important role in central tonic and baroreflex control of blood pressure, they may be potential neurotransmitters in the baroreflex control of ventilation.

RATIONALE FOR EXPERIMENTS

Data from a previous study I had performed indicated that the baro-pressure and baro-ventilatory reflex patterns obtained by a ramp pressure stimulation of the isolated carotid baroreceptors of the dog are different [32]. The results suggested that different central mechanisms or pathways mediate these responses. As presented in

the literature review, neural structures in the rostral ventrolateral medulla were known to affect vasomotor tone as well as ventilation, and specifically the role of the RVLM neural structures of the dog in maintaining ventilation had been investigated [1]. It was also known that the RVLM neural structures in the rat were part of the baro-pressure pathway [54]. This literature thus suggested that neural structures in the ventrolateral medulla may provide an explanation for the dichotomy in the baro-pressure and baro-ventilatory responses to the ramp pressure stimuli to the carotid baroreceptors. Hence, it appeared appropriate to test the hypothesis that the RVLM neural structures of the dog (corresponding to the ventrolateral pressor area (VLPA), see the literature review) not only maintain vasomotor tone and ventilation but are also part of the pathways for the baro-pressure and baro-ventilatory reflexes. This hypothesis was tested by determining the baroreflex responses to carotid baroreceptor stimulation before and after focal cold block of neural structures in the RVLM (Chapter III). The attenuation or elimination of the baroreflex responses after focal cold block showed that the RVLM in the dog plays a role in mediating these reflexes.

Though the RVLM neural structures in the dog were found to be substrates for the baroreflexes, other regions near the ventral medullary surface, as well as deeper in the ventrolateral medulla, could also play a part in these reflexes. This idea was further supported by studies in cats, rats, and rabbits which described alterations in both ventilation and arterial pressure by VMS applications of drugs or cold blockade, or by microinjections of drugs into the caudal ventrolateral medullary depressor area

(VLDA, see literature review). Thus, the hypothesis was proposed that neural structures near the ventral medullary surface not only affect steady state arterial pressure and ventilation but also participate in the baroreceptor mediated inhibition of both systemic arterial pressure and ventilation. The lack of sufficiently detailed information regarding neural structures within the dog medulla, and the technical difficulty of the ventral approach to neural structures near the medullary surface and deeper in the ventrolateral medulla prompted us to use rats as the experimental animal for further studies.

There exist several other advantages of using the rat model. Previous studies on central pathways and anatomical substrates involved in the proposed research have been performed. Detailed stereotaxic maps of the central organization of the medulla for this species are available and the rat model also provides the advantage of genetic and size uniformity of brains for histological verification. The aortic baroreceptor stimulation can be mimicked by electrically stimulating the aortic depressor nerve. Additionally, this is a low cost model with potential use as a pathophysiological paradigm.

Thus, to test the hypothesis that neural structures near the VMS mediate the baro-pressure and baro-ventilatory reflexes, experiments were performed where the baroreflexes were studied before and after depression or excitation of the activity of neural structures near the VMS (Chapter IV). Some of the neural structures underlying the region of the VMS considered the "pressor area" have a tonic

GABAergic inhibition [33,61,63]. Hence, as a follow up to the previous study, we determined if these neural structures with GABA_A receptors affect ventilation and the baroreceptor reflexes (Chapter V).

Surface applications of drugs led to the conclusion that the baro-ventilatory reflex is not affected by superficial neural structures. Since deeper neural structures may not be reached by surface applications, microinjection techniques were utilized to determine the role of deeper neural structures in cardio-respiratory control.

In the past several years, microinjection of drugs has been used to chemically block receptors on neural structures. The effect has been shown to be localized to a few 100 microns of the site of drug injection permitting selective activation of neural structures at any depth from the VMS.

Considering the above reasons for using the microinjection technique, and because the role of the CVLM neural structures in mediating the baro-ventilatory responses had not been tested, experiments were done to test the hypothesis that neural structures of the rat CVLM (corresponding to the VLDA) play a role in baroreflex cardio-respiratory control (presented in Chapter VI). The role of excitatory amino acids receptors in the CVLM in mediating the cardio-respiratory responses were also determined.

REFERENCES

1. Adams, M., T. Chonan, N.S. Cherniack, and C. vonEuler. Effects on respiratory pattern of focal cooling in the medulla of the dog. *J. Appl. Physiol.*, 65(5):2004-2010 (1988).
2. Agarwal, S.K., A.J. Gelsema, and F.R. Calaresu. Neurons in rostral VLM are inhibited by chemical stimulation of caudal VLM in rats. *Am. J. Physiol.* 257:R265-R270 (1989).
3. Agarwal, S.K., A.J. Gelsema, and F.R. Calaresu. Inhibition of rostral VLM by baroreceptor activation is relayed through caudal VLM. *Am. J. Physiol.* 258:R1271-R1278 (1990).
4. Arata, A., H. Onimaru, and I. Homma. Respiration-related neurons in the ventral medulla of newborn rats in vitro. *Brain Res. Bull.*, 24:599-604 (1990).
5. Blessing, W.W. and J.O. Willoughby. Depressor neurons in rabbit caudal medulla do not transmit the baroreceptor-vasomotor reflex. *Am. J. Physiol.*, 253(22):H777-H786 (1987).
6. Bonham, A.C. and I. Jeske. Cardiorespiratory effects of DL-homocysteic acid in caudal ventrolateral medulla. *Am. J. Physiol.*, 256(25):H688-H696 (1989).
7. Botros, S.M. and E.N. Bruce. Neural network implementation of a three-phase model of respiratory rhythm generation. *Biol. Cybern.*, 63:143-153 (1990).
8. Brody, M.J., R.H. Alper, T.P. O'Neill, and J.P. Porter. Central neural control of the cardiovascular system. In: A. Zanchetti and R.C. Tarazi (eds.), *Handbook of Hypertension*, Elsevier Science Pub. B.V., Amsterdam, Vol. 8, p.1. (1986).
9. Brown, L.D. and P.G. Guyenet. Electrophysiological study of cardiovascular neurons in the rostral ventrolateral medulla in rats. *Circ. Res.*, 56:359-369, (1985).
10. Brunner, M.J., M.S. Sussman, A.S. Green, and A.K. Shoukas. Carotid sinus baroreceptor reflex control of respiration. *Circ. Res.*, 51:624-636, (1982).
11. Budzinska, K., C. von Euler, F.F. Kao, T. Pantaleo, and Y. Yamamoto.

Effects of graded focal cold block in rostral areas of the medulla. *Acta. Physiol. Scand.*, 124:329-340 (1985).

12. Ciriello, J., M.M. Caverson, and C. Polosa. Function of the ventrolateral medulla in the control of the circulation. *Brain Res. Rev.*, 11:359-391 (1986).
13. Dampney, R.A.L. Functional organization of central cardiovascular pathways. *Clin. Exp. Pharmac. Physiol.*, 8:241-259 (1981).
14. Dev, N.B. and H.H. Loeschcke. Topography of the respiratory and circulatory responses to acetylcholine and nicotine on the ventral surface of the medulla oblongata. *Pflüg. Arch.*, 379:19-27 (1979).
15. Donohue, S., R.B. Felder, D. Jordan and K.M. Spyer. The central projections of carotid baroreceptors and chemoreceptors in the cat: A neurophysiological study. *J. Physiol.*, 347:397-401 (1984).
16. Eldridge, F.L. and D.E. Millhorn. Central regulation of respiration by endogenous neurotransmitters and neuromodulators. *Ann. Rev. Physiol.*, 43:121-135 (1981).
17. Ellenberger, H.H. and J.L. Feldman. Monosynaptic transmission of respiratory drive to phrenic motoneurons from brainstem bulbospinal neurons in rats. *J. Comp. Neurol.*, 269:47-57 (1988).
18. Ellenberger, H.H. and J.L. Feldman. Brainstem connections of the rostral ventral respiratory group of the rat. *Brain Research*, 513:35-42 (1990).
19. Ellenberger, H.H., J.L. Feldman, and W.Z. Zhan. Subnuclear organization of the lateral tegmental field of the rat. II: Catecholamine neurons and ventral respiratory group. *J. Comp. Neurol.*, 294:212-222 (1990).
20. Euler, C. VON. Brain-stem mechanisms for generation and control of breathing pattern. In: *Handbook of Physiology. The Respiratory system. Control of Breathing*. Bethesda, MD: Am. J. Physiol. Soc., sect. 3, vol II, chapt. 9, p.463-523 (1986).
21. Feldman, J.L. and J.C. Smith. Cellular mechanisms underlying modulation of breathing pattern in mammals. *Ann. N. Y. Acad. Sci.*, 563:114-130 (1989).
22. Feldman, J.L., J.C. Smith, D.R. McCrimmon, H.H. Ellenberger, D.F. Speck.

- Generation of respiratory pattern in mammals. In A.M. Cohen, S. Grillner, and S. Rossignol (eds.): *Neural Control of Rhythmic Movements in Vertebrates*. New York: Wiley and Sons, chapt. 3, p.73-100 (1988).
23. Gardiner, S.M. and T. Bennett. Endogenous vasopressin and baroreflex mechanisms. *Brain Research*, 11:317-334 (1986).
 24. Gatti, P.J., W.P. Norman, A.M. Taveira Dasilva, and R.A. Gillis. Cardiorespiratory effects produced by microinjecting L-glutamate acid into medullary nuclei associated with the ventral surface of the feline medulla. *Brain Research*, 381:281-288 (1986).
 25. Gordon, F.J. Aortic baroreceptor reflexes are mediated by NMDA receptors in caudal ventrolateral medulla. *Am. J. Physiol.*, 252(21):R628-R633 (1987).
 26. Granata, A.R., D.A. Ruggiero, D.H. Park, T.H. Joh, and D.J. Reis. Brain stem area with C1 epinephrine neurons mediates baroreflex vasodepressor responses. *Am. J. Physiol.*, 248(17):H547-H567 (1985).
 27. Gurtzenstein, P.G. and O.U. Lopes. Cardiovascular responses evoked from the nicotine sensitive area on the ventral surface of the medulla oblongata in the cat. *J. Physiol.*, 347:345-360 (1984).
 28. Guyenet, P.G., T.M. Filtz, and S.R. Donaldson. Role of excitatory amino acids in rat vagal and sympathetic baroreflexes. *Brain Research*, 407:272-284 (1987).
 29. Housley, G.D. and J.D. Sinclair. Localization by kainic acid lesions of neurons transmitting the carotid chemoreceptor stimulus for respiration in the rat. *J. Physiol.*, 406:99-114 (1988).
 30. Huang, Q. and W.M. St. John. Respiratory neural activities after caudal-to-rostral ablation of medullary regions. *J. Appl. Physiol.* 64(4):1405-1411 (1988).
 31. Huang, Z.S., G.L. Gebber, S.M. Barman, and K.J. Varner. Forebrain contribution to sympathetic nerve discharge in anesthetized cats. *Am. J. Physiol.*, 252(21):R645-R652 (1987).
 32. Jung, R. and P.G. Katona. Cardiovascular and respiratory responses to slow ramp carotid sinus pressures in the dog. *J. Appl. Physiol.*, 68(4):1465-1474

(1990).

33. Keeler, J.R., C.W. Shults, T.N. Chase, and C.J. Helke. The ventral surface of the medulla in the rat: Pharmacological and autoradiographic localization of GABA-induced cardiovascular effects. *Brain Research*, 297:217-224 (1984).
34. Kubo, T. and M. Kihara. N-Methyl-D-aspartate receptors mediate tonic vasopressor control in the caudal ventrolateral medulla of the rat. *Brain Research*, 451:366-378 (1988).
35. Kubo, T. and M. Kihara. Evidence for γ -aminobutyric acid receptor mediated modulation of aortic baroreceptor reflex in the nucleus tractus solitarii of the rat. *Neurosci. Lett.*, 89:156-160 (1988).
36. Lawing, W.L., D.E. Millhorn, D.A. Bayliss, J.B. Dean, and A. Trzebski. Excitatory and inhibitory effects on respiration of L-glutamate microinjected superficially into the ventral aspects of the medulla oblongata in cat. *Brain Research*, 435:322-326 (1987).
37. Lipski, J., R.M. McAllen, and K.M. Spyer. The sinus nerve and baroreceptor input to the medulla of the cat. *J. physiol.*, 251:61-78 (1975).
38. Long, S.E. and J. Duffin. The medullary respiratory neurons: a review. *Can. J. Physiol. Pharmacol.*, 622:161-182 (1984).
39. Luft, F.C., Th. Unger, R.E. Lang, and D. Ganten. Chemical messengers and transmitters in the brain and cardiovascular regulation. In: A. Zanchetti and R.C. Tarazi (eds.), *Handbook of Hypertension*, Elsevier Science Pub. B.V., Amsterdam, Vol. 8, p.26. (1986).
40. Maass-Moreno, R. and P.G. Katona. Species dependence of baroreceptor effects on ventilation in the cat and the dog. *J. Appl. Physiol.*, 67:2116-2124 (1989).
41. Marson, L. and A.D. Loewy. Topographic organization of substance P and monoamine cells in the ventral medulla of the cat. *J. Auton. Nerv. System*, 14:271-285 (1985).
42. McAllen, R.M. Location of neurones with cardiovascular and respiratory function, at the ventral surface of the cat's medulla. *Neurosci.*, 18(1):43-49 (1986).

43. McWilliam, P.N. and S.L. Shepherd. A GABA-mediated inhibition of neurones in the nucleus tractus solitarius of the cat that respond to electrical stimulation of the carotid sinus nerve. *Neurosc. Lett.*, 94:321-326 (1988).
44. Mitchell, R.A., H.H. Loeschcke, W.H. Massion, and J.W. Severinghaus. Respiratory responses mediated through superficial chemosensitive areas on the medulla. *J. Appl. Physiol.*, 18(3):523-533 (1963).
45. Millhorn, D.E. and F.L. Eldridge. Role of ventrolateral medulla in regulation of respiratory and cardiovascular systems. *J. Appl. Physiol.*, 61(4):1249-1263 (1986).
46. Miura, M., K. Takayama, and J. Okada. Study of possible transmitters in the solitary tract nucleus of the cat involved in the carotid sinus baro- and chemoreceptor reflex. *J. Auton. Nerv. Sys.*, 19:179-188 (1987).
47. Moss, I.R., M. Denavit-Saubié, F.L. Eldridge, R.A. Gillis, M. Herkham, and S. Lahiri. Neuromodulators and transmitters in respiratory control. *Fed. Proc.* 45:2133-2147 (1986).
48. Onai, T., M. Saji, and M. Miura. Projections of supraspinal structures to the phrenic motor nucleus in rats studied by a horseradish peroxidase microinjection method. *J. Auton. Nerv. Sys.*, 21:233-239 (1987).
49. Onai, T., M. Saji, and M. Miura. Functional subdivisions of the nucleus tractus solitarii of the rat as determined by circulatory and respiratory responses to electrical stimulation of the nucleus. *J. Auton. Nerv. Sys.*, 21:195-202 (1987).
50. Onimaru, H., A. Arata, and I. Homma. Primary respiratory rhythm generator in the medulla of brainstem-spinal cord preparation from newborn rat. *Brain Research*, 445:314-324 (1988).
51. Onimaru, H., A. Arata, and I. Homma. Electrophysiological properties and localization of inspiratory pattern generator in medulla isolated from newborn rats. *Jpn. J. Physiol.*, 39:S249 (1989).
52. Richter, D.W. Generation and maintenance of the respiratory rhythm. *J. Exp. Biol.*, 100:93-107 (1982).
53. Richter, D.W. and H. Sellar. Baroreceptor effects on medullary respiratory neurones of the cat. *Brain Research*, 86:168-171 (1975).

54. Ross, C.A., D.A. Ruggiero, D.H. Park, T.H. Joh, A.F. Sved, J. Fernandez-Pardal, J.M. Saavedra, and D.J. Reis. Tonic and vasomotor control by the rostral ventrolateral medulla: Effect of electrical or chemical stimulation of the area containing C1 adrenalin neurons on arterial pressure, heart rate and plasma catecholamines and vasopressin. *J. Neurosc.* 4(2):474-494 (1984).
55. Ross, C.A., D.A. Ruggiero, and D.J. Reis. Projections from the nucleus tractus solitarii to the rostral ventrolateral medulla. *J. Comp. Neurol.*, 242:511-534 (1985).
56. Saether, K., G. Hillaire, R. Monteau. Dorsal and ventral respiratory groups of neurons in the medulla of the rat. *Brain Research*, 419:87-96 (1987).
57. Saji, M. and M. Miura. Thoracic expiratory motor neurons of the rat: localization and sites of origin of their premotor neurons. *Brain Research*, 507: 247-253 (1990).
58. Sapru, H.N. and A.J. Krieger. Carotid and aortic chemoreceptor function in the rat. *J. Appl. Physiol.*, 42(3):344-348 (1977).
59. Schmid, K., G. Böhmer, and K. Gebauer. GABA_B receptor mediated effects on central respiratory system and their antagonism by phaclofen. *Neurosc. Lett.*, 99:305-310 (1989).
60. Urbanski, R.W. and H.N. Sapru. Evidence for a sympathoexcitatory pathway from the nucleus tractus solitarii to the ventrolateral medullary pressor area. *J. Auton. Nerv. Sys.* 23:161-174 (1988).
61. Urbanski, R.W. and H.N. Sapru. Putative neurotransmitters involved in medullary cardiovascular regulation. *J. Auton. Nerv. Sys.* 25:181-193 (1988).
62. Willette, R.N., A.J. Krieger, P.P. Barcas, and H.N. Sapru. Medullary γ -aminobutyric acid (GABA) receptors and the regulation of blood pressure in the rat. *J. Pharmacol. Exp. Therap.*, 226(3):893-899 (1983).
63. Willette, R.N., S. Punnen, A.J. Krieger, and H.N. Sapru. Interdependence of rostral and caudal ventrolateral medullary areas in the control of blood pressure. *Brain Research*, 321:169-174 (1984).
64. Yamada, K.A., W.P. Norman, P. Hamosh, and R.A. Gillis. Medullary ventral surface GABA receptors affect respiratory and cardiovascular function. *Brain*

Research, 248:71-78 (1982).

Fig. 1 Schematic representation for baro-pressure pathways. CVLM - Caudal ventrolateral medulla, GR - GABAergic receptor sites, GLU - Glutamatergic receptor sites, IML - Intermediolateral column, NTS - Nucleus Tractus Solitarius, RVLM - Rostral ventrolateral medulla, VLDA - Ventrolateral Depressor Area, VLPA - Ventrolateral Pressor Area, + excitatory, - inhibitory.

CHAPTER III¹
EFFECTS ON BAROREFLEX CONTROL OF ARTERIAL PRESSURE AND
VENTILATION OF FOCAL COOLING IN THE ROSTRAL MEDULLA OF
THE DOG

INTRODUCTION

It is well known that the cardiovascular and respiratory systems interact as is evidenced by their responses to hypercapnia, hypoxia and exercise. However, the neurophysiological mechanisms, anatomical substrates, and pathways involved in the central interaction of these two systems remain to be determined. While it is likely that certain major areas of the brainstem subserve strictly respiratory or cardiovascular functions, there is increasing evidence that some areas may be involved in the control of both systems [3,13,18].

Arterial baroreceptor stimulation inhibits both arterial pressure and ventilation [11] thereby providing a tool for investigating the role of central neural structures in integrative cardio-respiratory control. Neurons in the rostral ventrolateral medulla have been implicated in vasomotor control and mediation of the arterial baroreflex [2,5,8,10,17]. Stimulating or lesioning areas in the ventral medulla which evoke arterial pressure responses overlap areas which, when disturbed, alter respiration [9,12,16,18,19]. Specifically, it has been suggested that neural structures in the rostral

¹ Supported in part by NIH Program Project Grant HL-2283007.

ventrolateral medulla (RVLM) play an integrative role [3,18]. Hence, the goal of this study was to test the hypothesis that neural structures in the RVLM of the dog that affect control of vasomotor tone and ventilation, also mediate the baroreflex inhibition of arterial pressure ('baro-pressure' reflex) and ventilation ('baro-ventilatory' reflex).

METHODS

Experimental techniques and surgical preparation

Six dogs (10-14 Kg) were anesthetized with sodium pentobarbital (35 mg/Kg; i.v.), intubated and artificially ventilated with 100% O₂. Cannulas were placed in the femoral artery and vein to measure systemic arterial pressure and to infuse drugs, respectively. Arterial blood gases and pH were obtained periodically (Corning 175), and the latter was maintained between 7.35 and 7.45. A thermistor placed in the mid-thoracic esophagus was monitored to maintain core body temperature at $36 \pm 1^{\circ}\text{C}$. Expired CO₂ was monitored continuously (Beckman LB-2) via a tracheal catheter.

A pneumothorax was created by an incision in the lower intercostal space. The diaphragm was accessed and the electromyogram (EMG) recorded with twisted wire electrodes to obtain respiratory muscle electrical activity. The EMG was amplified, band pass filtered (20-2000 Hz), rectified and processed through a third order Paynter filter ($\tau = 200$ msec).

The vago-sympathetic trunks were exposed on both sides through a ventral mid-

cervical incision and sectioned. The common carotid arteries and the carotid sinus regions were also exposed bilaterally and each carotid sinus region isolated into a 'blind - sac' by ligating the thyroid, ascending pharyngeal, laryngeal, internal carotid, occipital and external carotid arteries as previously described [11]. Intact vertebral arteries in the dog are sufficient to provide adequate perfusion of the brain [7]. The occipital arteries were carefully dissected and ligated at their origin to prevent pressure changes in the isolated carotid sinuses from affecting the carotid bodies [11]. The common carotid arteries were tied proximal to the heart and the distal ends of both vessels cannulated using wide bore tubing connected as a 'Y' and filled with heparinized saline (50 U/ml). The common end of the 'Y' was connected to a bi-directional syringe pump (Harvard Apparatus). The position of the plunger was adjusted to provide a steady-state systemic arterial pressure (SAP) at a carotid sinus pressure (CSP) of 50 mmHg.

All animals were placed prone and the head fixed in a horizontal plane in a stereotaxic frame (David Kopf). The dorsal surface of the brain stem was exposed by an occipital craniotomy and the dura cut [1]. Small regions of neural tissue were selectively blocked unilaterally using a "Cryolab 3" (Bertin). The probe construction, operation and placement details have been detailed previously [1]. Using the obex as the reference point, the cryoprobe (0.9 mm diameter needle tip) was inserted into the brainstem at 45° from the Horsley-Clarke horizontal plane. A 1 mm grid system was used to place the probe. Data in a previous study [6] indicated that mean arterial

pressure decreased when focal cooling was done at sites in the rostral ventrolateral medulla, therefore the probe tip was positioned at similar sites.

Protocol

The baseline mean arterial pressure (MAP in mmHg), peak integrated diaphragmatic activity (Edi in arbitrary units), respiratory frequency (F in breaths/min), and ventilation (Vn in arbitrary units; product of F and Edi), and baro-pressure and baro-ventilatory reflex curves were determined before and after unilateral focal cold block. To obtain the baroreflex curves, at each site after probe placement and with the tip at body temperature, the CSP was first maintained at 50 mmHg for at least 3 minutes. Then to obtain the baro-pressure (6 dogs) and baro-ventilatory reflex curves CSP was changed from 50 mmHg to 200 mmHg as a 1 mmHg/sec ramp produced by saline infusion. Technical problems in recording EMG's prevented obtaining the baro-ventilatory reflex in one dog. Using a ramp pressure minimizes the time required to characterize the baroreflex over the entire pressure sensitive range of the baroreceptors [11]. After a trial with the probe tip at body temperature, the CSP was returned to 50 mmHg for 3 minutes, after which the probe tip was cooled to 13-16°C to obtain focal cold block. With the probe tip cooled all variables were again determined.

At the end of the experiment the brainstem was removed and fixed in buffered formalin and sectioned. Serial paraffin sections (20 μ thick) were obtained. They were inspected under a light microscope and the sections with lesions formed by the

probe placement determined. These sections were stained with hematoxylin and eosin and mounted. After locating the tip of the probe (the last serial section with a lesion) and determining the neural structures surrounding the tip by comparison with a stereotaxic dog atlas [14], the sites were marked on projected outlines of the sections.

Data analysis

The SAP, CSP, end tidal PCO_2 , and the moving average of the diaphragmatic EMG were recorded on a strip chart recorder (Electronics for Medicine, Inc.). The baseline values for MAP, Edi, F and Vn before and after cooling were compared by paired Student's t-tests. For analysis of the baroreflexes, the MAP and average F and Edi after every 10 mmHg increase in CSP were determined. The Vn, F, and Edi data were then smoothed using a 3-point averager. Each variable was also expressed as a percentage of its control value at a CSP of 50 mmHg.

For the baro-pressure reflex curves threshold was defined as the CSP at which MAP had decreased by at least 2% from its value at a CSP of 50 mmHg, and saturation was considered to have occurred when MAP no longer decreased by more than 2%. Similar definition was used for the threshold of the baro-ventilatory reflex. Saturation, however, was defined as the CSP at which Vn had stopped decreasing by less than 2% or had started increasing. The non-monotonic behavior of the baro-ventilatory reflex that necessitated this definition has been discussed in a previous study [11]. Linear regression with ANOVA was used to fit a straight line to the MAP

or V_n values between threshold and saturation. The slopes of the regression lines obtained before and after cooling were compared using the method described in [12]. These slopes indicate the baro-pressure gain, G_{MAP} , and the baro-ventilatory gain, G_{Vn} . Student's t-tests were used to determine if the decrease in MAP, V_n , and F between threshold and saturation on baroreceptor stimulation was significant. Paired student's t-tests were used for comparing the baseline values for the variables before and after cold block. Mean $\pm$ standard error of the mean are reported and comparisons considered significant for $P \leq 0.05$.

RESULTS

Figure 1 shows the histological locations of the sites of focal cold block. Five sites were located about 5 mm rostral to the obex while one was about 7 mm rostral to the obex. All were near the facial nucleus.

Figure 2 shows the effect of focal cold blockade on SAP and processed diaphragmatic activity in one dog. Table 1 gives a summary on a percentage basis of the change in MAP, Edi, F and V_n baseline values due to focal cooling. On average, with the CSP held at 50 mmHg, focal cooling caused the baseline MAP to drop significantly from 128.6 ± 2.6 mmHg to 96.8 ± 6.6 mmHg (net decrease of $24.7 \pm 4.8\%$). Concurrent with the reduction in MAP, V_n did not change significantly ($P = 0.7$) though F increased significantly from 15.5 ± 3.6 breaths/min to 23.9 ± 7.3 breaths/min (net increase of $55.0 \pm 19.8\%$) and Edi decreased significantly by $28.7 \pm$

7.7%. At one site (included in the average) there was no change in Edi and F decreased by 24.6%.

Table 2 gives the change in MAP and Vn between threshold and saturation due to baroreceptor stimulation before and after cooling. The ramp increase of CSP from 50 to 200 mmHg caused the MAP to decrease significantly in all dogs with the probe tip at body temperature (Fig. 3a); the average reduction in MAP being 43.7 ± 3.4 mmHg ($34.5 \pm 2.6\%$). With the probe tip cooled, raising the CSP again caused the MAP to decrease significantly at all sites on average by 20.0 ± 5.2 mmHg ($20.0 \pm 4.8\%$; Fig. 3a).

The MAP at saturation was higher before focal cold block (82.8 ± 3.7 mmHg) than after (75.4 ± 4.6 mmHg). The depressor effect of focal cold block was generally greater when the background MAP was high than when it was low. Hence, baro-pressure gain G_{MAP} , i.e., the change in MAP for a given change in CSP, was reduced after focal cold block from -0.56 ± 0.09 to -0.28 ± 0.08 (a decrease of $47.3 \pm 14.6\%$). As shown in Fig. 4, the linear regression fit to the baro-pressure response curve between threshold and saturation was less steep after cooling, and in 5 dogs this diminution of slope was significant ($P < 0.05$ at each of these sites). In one dog, where there was no effect of cooling on Edi, the baro-pressure reflex gain was not significantly altered.

Without cooling the probe raising the CSP also inhibited ventilation in 4 out of 5 dogs (Fig. 3b, the 5th dog had a less than 2% inhibition in Vn). This decrease in

V_n was non-monotonic (a curve is considered to be monotonic in an interval if it is either nondecreasing, i.e., it goes up or stays the same, or nonincreasing, i.e., it goes down or stays the same, everywhere within that interval). After a significant inhibition between threshold and saturation ($13.7 \pm 3.7\%$), V_n started increasing even though the CSP was still being increased; Fig. 3b. The respiratory frequency also decreased as CSP increased in a non-monotonic fashion, the decrease in F ($12.6 \pm 4.5\%$) being significant; Fig. 3c. Edi , however tended to increase as CSP increased Fig. 3d. The increase in Edi occurred mainly at higher CSP levels at which time the inhibitory action on frequency was also paradoxically reversed, and thus the inhibition of ventilation by baroreceptor stimulation was also reversed.

Focal cooling depressed the baro-ventilatory response. Raising the CSP from 50 mmHg to 200 mmHg did not cause a significant reduction in V_n ($4.5 \pm 2.7\%$; $P = 0.2$) or F ($7.0 \pm 4.1\%$; $P = 0.16$). The baro-ventilatory gain G_{v_n} , i.e. change in ventilation for a given change in CSP was significantly reduced after focal cold block in the 4 dogs that showed a response. The fifth dog that did not show any baro-ventilatory response before cooling again did not respond to baroreceptor stimulation. The linear regression fits to the baro-ventilatory curves between threshold and saturation are shown in Fig. 5, and on average G_{v_n} decreased by $78.9 \pm 13.6\%$. In 2 dogs the tendency for Edi to rise at higher CSP was abolished. However, in two other dogs (including the one in which Edi did not decrease on cooling and the baro-pressure gain had not changed significantly on cooling) the paradoxical rise in Edi

persisted after the cold block.

DISCUSSION

The results of this study not only confirm that neural structures of the rostral ventrolateral medulla of the dog affect vasomotor tone and ventilation but also show that the RVLM neural structures of the dog play a role both in the baroreflex inhibition of blood pressure and ventilation.

Previous studies using focal cooling in the dog RVLM produced a decrease in peak diaphragmatic activity, often with increased respiratory frequency [1,6]. Similar findings have been reported in cats [4]. We also observed a similar depression in diaphragmatic activity with an increased respiratory rate in our experiments. Concomitant with the respiratory effects on focal cooling we also saw a decrease in mean arterial pressure indicating that the cooled area was part of the neural substrate for maintaining vasomotor tone, in addition to being involved in maintaining tidal volume. Similar simultaneous responses of arterial pressure and ventilation have also been observed by the graded cooling of the ventral medullary surface (VMS) in cats and rats [19,20,21], and on focal cooling of the rostral medulla in cats [4].

The regions of the RVLM that maintain vasomotor tone in the cat and the rat have also been suggested to be part of the sympatho-inhibitory baroreflex pathway [2,5,8,10]. Unlike in other studies, in this study the baroreceptors were stimulated over their entire range of sensitivity. Focal cooling caused not only a decrease in the

baseline MAP but it also decreased the baro-pressure reflex gain. Thus in the dog, as in other species, the RVLM plays a role in the baroreceptor control of systemic arterial pressure.

Accompanying the baroreflex inhibition of MAP was the baroreflex inhibition of ventilation. The decrease in arterial pressure and ventilation were observed to be less than we have reported previously in similar experiments done on spontaneously breathing anesthetized dogs [11]. The attenuation in the present experiments could have been because of depression of the cardio-respiratory system of the dogs due to anesthetic levels or because of tissue damage during probe placement. The baro-ventilatory responses observed, however, did show the non-monotonic behavior that we have described earlier [11]. We have previously speculated that the reversal in the ventilatory inhibition as the CSP is increased may be caused, in part, by central chemoreceptor excitation, elicited by the initial respiratory depression generated increase in the arterial PCO_2 . However, chemoreceptor reflex mechanisms could only explain part of this reversal, and hence it was suggested that differences in central neural mechanisms governing the baro-pressure and baro-ventilatory reflexes were likely [11]. In this study, the animals were artificially ventilated, thereby keeping PCO_2 constant during the carotid baroreceptor stimulation. However, the non-monotonic baro-ventilatory reflex persisted. This suggests that mechanisms other than chemoreceptor excitation are responsible for the paradoxical reversal of the baro-ventilatory reflex. On focal cooling the RVLM, not only was the initial inhibition of

ventilation attenuated, but in two of the dogs the paradoxical increase in Edi was also blocked. Therefore, the neural substrates in the RVLM that were cooled in these experiments are not only part of the baro-ventilatory pathway but may also be responsible for the differences in the baro-pressure and baro-ventilatory reflexes.

Although a possible contribution of dorsal medullary pathways to the baro-ventilatory reflex cannot be ruled out, the present results show that neural structures in the RVLM are part of the baro-ventilatory pathway. The baro-ventilatory reflex could be mediated by direct inhibitory projections from the nucleus tractus solitarius (site of first synapse of the baroreceptor afferents [15]) to neurons that are part of the ventral respiratory group. Alternatively, it could be mediated via connections from the intermingled cardiovascular neurons of the baro-pressure pathway that are present in this region.

In one dog, neither the baseline Edi decreased on cooling, nor did the paradoxical rise in Edi on baroreceptor stimulation get abolished. The baro-pressure gain was also not attenuated significantly on focal cooling. Histological verification of the cooling site indicated that it was rostral to all other sites and this may account for the anomalous responses.

In summary, the rostral ventrolateral medullary structures of the dog near the facial nucleus maybe part of the baro-ventilatory pathway, besides being part of the baro-pressure pathway. These neural structures not only affect the baroreflex control of arterial pressure and ventilation but also maintain baseline arterial pressure and

ventilation. This suggests that the RVLM plays an important role in integrative cardio-respiratory control.

REFERENCES

1. Adams, M., T. Chonan, N.S. Cherniack, and C. von Euler. Effects on respiratory pattern of focal cooling in the medulla of the dog. *J. Appl. Physiol.*, 65(5):2004-2010 (1988).
2. Agarwal, S.K, A.J. Gelsema, and F.R. Calaresu. Inhibition of rostral VLM by baroreceptor activation is relayed through caudal VLM. *Am. J. Physiol.*, 27:R1271-R1278 (1990).
3. Bruce, E.N. and N.S. Cherniack. Central chemoreceptors. *J. Appl. Physiol.*, 62:389-402 (1987).
4. Budzinska, K., C. von Euler, F.F. Kao, T. Pantaleo, and Y. Yamamoto. Effects of graded focal cold block in rostral areas of the medulla. *Acta. Physiol. Scand.*, 124:329-340 (1985).
5. Caverson, M.M., J. Ciriello, and F.R. Calaresu. Chemoreceptor and baroreceptor inputs to ventrolateral medullary neurons. *Am. J. Physiol.*, 247(16):R872-R879 (1984).
6. Chonan, T., E.M. Adams, C.v. Euler, and N.S. Cherniack. Effects of focal cooling in the ventrolateral medulla on chemoresponsiveness in dogs. *Resp. Physiol.*, 80:45-54 (1990).
7. Chungcharoen, D., M. DeBurgh Daly, E. Neil, and A. Schweitzer. The effect of carotid occlusion upon the intrasinus pressure with special reference to vascular communications between the carotid and vertebral circulations in the dog, cat, and rabbit. *J. Physiol.*, 117:56-76 (1952).
8. Ciriello, J., M.M. Caverson, and C. Polosa. Function of the ventrolateral medulla in the control of the circulation. *Brain. Res. Rev.*, 11:359-391 (1986).
9. Gatti, P.J., W.P. Norman, A.M. Taveira Dasilva, and R.A. Gillis. Cardiorespiratory effects produced by microinjecting L-Glutamic acid into medullary nuclei associated with the ventral surface of the feline medulla. *Brain. Res.*, 381:281-288 (1986).
10. Granata, A.R., D.A. Ruggiero, D.H. Park, T.H. Joh, and D.J. Reis. Brain stem area with C1 epinephrine neurons mediates baroreflex vasodepressor responses. *Am. J. Physiol.*, 248(17):H547-H567 (1985).

11. Jung, R. and P.G. Katona. Cardiovascular and respiratory responses to slow ramp carotid sinus pressures in the dog. *J. Appl. Physiol.*, 68(4):1465-1474 (1990).
12. Kleinbaum, D.G. and L.L. Kupper. *Applied regression analysis and other multivariable methods*. PWS, Publishers; Boston, Massachusetts, 1978.
13. Koepchen, H.P. Respiratory and cardiovascular "centers": Functional entirety or separate structures? In: *Central neurone environment and the control systems of breathing and circulation*. Springer-Verlag, Publisher; Heidelberg, 1983.
14. Lim, R.K.S., C.N.Liu and R.L. Moffitt. A stereotaxic atlas of the dog's brain. Charles C. Thomas, Publisher; Springfield, Ill., USA, 1960.
15. Lipski, J., R.M. McAllen, and K.M. Spyer. The sinus nerve and baroreceptor input to the medulla of the cat. *J. Physiol.*, 251:61-78 (1975).
16. McAllen, R.M. Location of neurones with cardiovascular and respiratory function, at the ventral surface of the cat's medulla. *Neuroscience*, 18(1):43-49 (1986).
17. McAllen, R.M., J.J.Neil, and A.D.Loewy. Effects of kainic acid applied to the ventral surface of the medulla oblongata on vasomotor tone, the baroreceptor reflex and hypothalamic autonomic responses. *Brain. Res.*, 238:65-76 (1982).
18. Millhorn, D.E. and F.L. Eldridge. Role of ventrolateral medulla in regulation of respiratory and cardiovascular systems. *J. Appl. Physiol.*, 61(4):1249-1263 (1986).
19. Mitchell, R.A., H.H. Loeschcke, W.H. Massion, and J.W. Severinghaus. Respiratory responses mediated through superficial chemosensitive areas on the medulla. *J. Appl. Physiol.*, 18(3):523-533 (1963).
20. Mitra, J., N.R. Prabhakar, J.L. Overholt, and N.S. Cherniack. Respiratory and vasomotor responses to focal cooling of the ventral medullary surface (VMS) of the rat. *Resp. Physiol.*, 74:35-48 (1988).
21. Schläfke, M. and H.H. Loeschcke. Lokalisation eines an der Regulation von Atmung und Kreislauf beteiligten Gebietes an der ventralen Oberfläche der Medulla oblongata durch Kalteblockade. *Pflugers Archiv.*, 297:201-220 (1967).

Table 1 Percentage change in baseline values of mean arterial pressure (MAP), ventilation (Vn), respiratory frequency (F), peak diaphragmatic activity (Edi) in 6 dogs on unilateral focal cooling.

MAP (%)	$-24.7 \pm 4.9^*$
Vn (%)	$+ 5.8 \pm 12.0$
F (%)	$+55.0 \pm 19.8^*$
Edi (%)	$-28.7 \pm 7.7^*$

Values are mean $\pm$ s.e.m.

* indicates significant at $P \leq 0.05$.

Table 2 Percentage change in MAP and Vn (Δ MAP and Δ Vn repectively) between threshold and saturation on baroreceptor stimulation before and after unilateral focal cold block in 6 dogs.

	BEFORE	AFTER
Δ MAP (%)	$34.6 \pm 2.6^*$	$20.0 \pm 4.8^{*@}$
Δ Vn (%)‡	$13.7 \pm 3.7^*$	$+4.5 \pm 2.7^@$

Values are mean $\pm$ s.e.m.

* indicates significant decrease at $P \leq 0.05$.

@ indicates significantly reduced after cold block at $P \leq 0.05$.

‡ n = 5

Fig. 1. Schematic cross sections of the dog brain-stem indicating sites at which focal cold block was achieved. All sites indicated by closed circles. NTS, nucleus of solitary tract; IO, inferior olivary nucleus; NP, nu. praepositus; 7, facial nucleus. (+5 to +7 mm rostral to obex).

Fig. 2. Systemic arterial pressure (SAP) and diaphragmatic activity (EMG), before (left panel) and approximately 3 minutes after cooling probe up to 16°C (right panel). ETCO₂, end tidal CO₂.

Fig. 3. Average baroreflex responses before and after focal cold block. a) Mean arterial pressure (MAP; $n=6$), b) Ventilation (V_n ; $n=5$), c) Frequency (F ; $n=5$), and d) Peak diaphragmatic activity (E_{di} ; $n=5$). Circles represent probe tip warm, triangles represent probe tip cold.

Fig. 4. Regression fits to the baro-pressure response curves for each animal. Regression lines represent fits to the data before (circles) and after cooling (triangles) the probe tip. Data points are actual values used for the regression fit. Mean arterial pressure (MAP) and Carotid sinus pressure (CSP) in mmHg.

Fig. 5. Regression fits to the baro-ventilatory response curves for each animal. Regression lines represent fits to the data before (circles) and after cooling (triangles) the probe tip. Data points are actual values used for the regression fit. Ventilation (V_n) in arbitrary units (a.u.) and Carotid sinus pressure (CSP) in mmHg.

CHAPTER IV^{1,2}

TONIC AND BAROREFLEX EFFECTS ON ARTERIAL PRESSURE AND VENTILATION OF PENTOBARBITAL AND NICOTINE ON THE RAT VENTRAL MEDULLARY SURFACE

INTRODUCTION

Arterial baroreceptor stimulation inhibits both arterial pressure and ventilation [3,10] (i.e, the "baro-pressure" and "baro-ventilatory" reflexes). Regions of the ventrolateral medulla (VLM) are involved not only in tonic vasomotor control and mediation of arterial baroreflexes, but also in respiratory control [1,7,8]. The goal of this study was to test the hypothesis that the neural structures in the VLM near the VMS that affect the tonic control of vasomotor tone and modulate ventilation [6,8], also mediate the baro-pressure and baro-ventilatory reflexes.

METHODS

Sprague-Dawley rats weighing 375-550 g were anesthetized with urethane (1.1 - 1.3 g/kg, i.p.). Rectal temperature was maintained at $36 \pm 1^\circ\text{C}$ with a heating pad and heating lamp. Cannulas in the femoral artery and femoral vein were used

¹ This chapter has been published as "Tonic and baroreflex effects on arterial pressure and ventilation of pentobarbital and nicotine on the rat ventral medullary surface", Brain Research, 485:399-402 (1989).

² This work was supported by NIH Program Project Grant HL25830.

respectively for measuring arterial pressure and for infusions. A ventral cervical midline incision was made and the trachea, carotid arteries, vagi, aortic depressor nerves, and carotid sinus nerves were exposed. The trachea was cannulated and the animals spontaneously breathed O₂ enriched room air. A pneumotachometer was used to derive the respiratory flow. Respiratory frequency (F) and tidal volume (V_T) were obtained by a computer algorithm that detected the beginning of inspiration and expiration from the respiratory flow. Ventilation ($\dot{V}_E$) was calculated as the product of tidal volume and respiratory frequency. With the rats in supine position, the larynx and oesophagus were reflected and the underlying musculature excised to expose the occipital bone which was removed to form a window approximately 6-7 mm long and 5-6 mm wide. The dura was cut and reflected to expose the ventral medullary surface.

The left (5 rats) or right (2 rats) aortic depressor nerve (ADN) was placed on Ag-AgCl bipolar stimulating electrodes and covered with petroleum jelly or mineral oil. In 2 of these rats additional experiments were performed with the contralateral ADN, vagi, and both carotid sinus nerves sectioned. In 2 other rats additional experiments were performed under partial denervation obtained by sectioning all the above mentioned nerves except the vagi (1 rat) or the carotid sinus nerves (1 rat).

Initially stimulus-response curves for arterial pressure and respiratory rate were obtained in 7 rats by stimulating the ADN with a stimulus of 3 or 5 volts and 2 or 0.2 msec duration, and varying the stimulation frequency from 2 to 120 pulses per second

(pps). After having determined a range for supramaximal stimulus parameters, all further experiments were done with supramaximal ADN stimulation with a train of square pulses at 3-5 volts, 80-120 pps, and 0.2 msec duration. The parameters for the pulse train were kept fixed for the entire experiment. To depress neural activity in the brain stem, sodium pentobarbital (16 or 32 mg/ml), which acts at GABA-ergic receptor sites [5], was applied in volumes of 1-4 μ l (diluted in mock cerebrospinal fluid (CSF)) to the VMS using small pieces of gelfoam approximately 2-3 mm square. Similarly, to provide respiratory stimulation, nicotine (10^{-4} g/ml), in volumes of 1-4 μ l (diluted in mock CSF) was applied to the VMS. The gelfoam pieces were centered unilaterally (ipsilateral to the ADN being stimulated) or bilaterally, approximately 2 mm lateral to the basilar artery. Rostrocaudally the position of the center ranged from 0 mm to approximately 3.5 mm rostral to the caudal edge of the occipital foramen. Arterial pressure and respiratory responses were obtained before and after applying pentobarbital or nicotine, and again after cleaning the VMS with warm mock CSF.

Rats were divided into 2 groups. Group 1 consisted of 5 rats with all buffer nerves intact and Group 2 consisted of 4 rats with complete or partial denervation of buffer nerves. The mean arterial pressure (MAP), and F, V_T , and $\dot{V}_E$ for each breath were determined and then stored by a computer. The data was then smoothed by a moving average of every 3-breaths. The baseline values were calculated by averaging this smoothed data over 5 breaths obtained before ADN stimulation was started to obtain the baseline MAP, F, V_T , and $\dot{V}_E$. For rats belonging to Group 1, Student's

t-tests were used for pairwise comparisons of (1) the baseline levels of MAP, F, V_T , and $\dot{V}_E$ before and after application of a drug on the VMS; (2) the maximum decrease in MAP, F, V_T , and $\dot{V}_E$ due to ADN stimulation obtained before and after drug application; and (3) the maximum decrease in MAP, F, V_T , and $\dot{V}_E$ obtained before applying the drug and after cleaning the VMS with mock CSF. The effects were considered significant for $P \leq 0.05$. All data is given as mean $\pm$ S.E.M..

RESULTS

Fig. 1 illustrates the responses of one of the 5 Group 1 rats to ADN stimulation. ADN stimulation reduced arterial pressure and respiration both before and after applying pentobarbital to the VMS. The absolute responses of arterial pressure and ventilation to ADN stimulation, for each of the 5 rats, under the three conditions are shown in Fig.2. Considering all 5 animals, before pentobarbital application, ADN stimulation reflexly lowered all variables significantly. On average, the reflex fall in MAP, $\dot{V}_E$, F, and V_T was $42.2 \pm 3.3\%$, $17.7 \pm 4.2\%$, $11.3 \pm 3.5\%$, and $7.3 \pm 2.2\%$, respectively. Application of pentobarbital to the VMS reduced the baseline MAP by 31.12 ± 4.8 mmHg, $\dot{V}_E$ by 30.67 ± 14.3 ml/min, F by 8.83 ± 6.4 breaths/min, and V_T by 0.12 ± 0.1 ml; all decreases in the control levels were significant. ADN stimulation now again caused a significant reflex reduction in MAP ($32.8 \pm 2.7\%$), $\dot{V}_E$ ($26.8 \pm 7.6\%$), F ($18.7 \pm 7.3\%$), and V_T ($10.4 \pm 2.2\%$). Pairwise comparisons indicated, however, that the reflex decrease in MAP after application of pentobarbital

was significantly less than that before applying pentobarbital, but no significant difference could be demonstrated in the reflex decrease of $\dot{V}_E$ ($P = 0.16$) and F ($P = 0.32$). The decrease in V_T was significantly enhanced. Comparison of the reflex responses obtained before applying pentobarbital to the VMS, and after cleaning it with mock CSF yielded no significant differences ($P > 0.1$ in all cases), indicating that the reflex changes were due to pentobarbital and not to spontaneous changes in the preparation. The same statistically-based conclusions were obtained when the reflex responses were considered as absolute responses. In 4 rats of Group 2, though no statistical comparisons were performed, the results were similar.

The reflex depression in arterial pressure and respiration to ADN stimulation were also compared before and after applying nicotine to the VMS of the 5 rats in Group 1. The absolute MAP and $\dot{V}_E$ responses obtained under the three conditions are illustrated in Fig. 3 for all 5 rats. Before nicotine application, ADN stimulation again depressed all variables significantly; on average the MAP, $\dot{V}_E$, F , and V_T decreased by $38.4 \pm 4.9\%$, $19.7 \pm 4.6\%$, $12.2 \pm 2.4\%$, and $8.7 \pm 2.8\%$, respectively. Nicotine application significantly reduced the baseline MAP by 18.2 ± 3.9 mmHg, and significantly raised $\dot{V}_E$ by 169.9 ± 52.3 ml/min, F by 62.4 ± 17.5 breaths/min, and V_T by 0.4 ± 0.1 ml. ADN stimulation still significantly depressed the four variables by $29.2 \pm 6.1\%$, $12.1 \pm 0.8\%$, $6.1 \pm 1.1\%$, and $6.4 \pm 0.8\%$, respectively. However, unlike the results for pentobarbital, a pairwise comparison between the reflex responses before and after application of nicotine on the VMS showed no statistically

significant difference for any variable ($P > .05$ for all variables). Pairwise comparisons of responses before applying nicotine and after cleaning the VMS with mock CSF failed to show any significant differences ($P > .2$), once again verifying the stability of the preparation. Again, the conclusions were unaltered by considering the responses in absolute levels. 3 rats of Group 2 in which statistical evaluation was not performed showed similar results.

DISCUSSION

The aortic depressor nerve of the rat has been shown to contain purely baroreceptor afferents [9], hence ADN stimulation is considered to mimic aortic baroreceptor afferent stimulation. In our preparation, there is a significant depression in ventilation and arterial pressure with and without the other buffer nerves intact. These results are in general agreement with those of Sapru et al. [10] who found inhibitory effects on arterial pressure and ventilation by ADN stimulation. However, while they reported a consistent cessation of breathing due to ADN stimulation, we only saw a depression in ventilation. This difference may be caused by their use of decerebrate rats and a different strain. We observed ventilatory cessation only if the superior laryngeal nerve was directly stimulated or there was current spread from an electrode placed on the ADN close to the superior laryngeal nerve. In order to determine the neural structures that are involved in the baro-pressure and baro-ventilatory reflexes, application of pentobarbital and nicotine to the VMS was used

to alter the functioning of the neural structures. The depression of arterial pressure and ventilation we observed on applying pentobarbital to the VMS was similar to the depression obtained by applying pentobarbital to the rostral glycine sensitive area of the VMS in the cat [4]. Similarly, the effect of nicotine on MAP and ventilation is similar to that which occurs on its application to the Mitchell and Loeschcke areas of the VMS in the cat [2].

Since we also obtained a depression of the baro-pressure reflex on applying pentobarbital to the VMS, the neural structures that are responsible for the decrease in MAP may be also a part of the baro-pressure reflex. This suggestion would have to be verified by ascertaining that the loss of baro-pressure reflex was not caused by the change in operating point (fall in baseline pressure level) following pentobarbital application. However, because nicotine did not affect the baro-pressure reflex, the nicotine sensitive neural structures affected probably do not play a role in this reflex. It was surprising that neither pentobarbital nor nicotine influenced the baro-ventilatory reflex inspite of their significant depressant and excitatory influences, respectively, on the control level of ventilation. The affected structures are thus unlikely to be a part of the baro-ventilatory reflex pathway.

In conclusion, vasomotor tone and ventilatory drive can be affected by neural structures of the ventrolateral medulla near the ventral surface in rats. While these structures may participate in the baro-pressure reflex, they are not involved in the baroreflex mediated control of ventilation. Further studies are needed to better

localize the neural structures responsible for tonic versus reflex control of arterial pressure and ventilation, and to investigate the apparent dichotomy in the neural organization for baro-pressure and baro-ventilatory control.

REFERENCES

1. Ciriello, J., M.M. Caverson, and C. Polosa, Function of the ventrolateral medulla in the control of the circulation. *Brain Res. Rev.*, 11:359-391 (1986).
2. Dev, N.B., H.H. Loeschcke, Topography of the respiratory and circulatory responses to acetylcholine and nicotine on the ventral surface of the medulla oblongata. *Pflügers Arch.*, 379:19-27 (1979).
3. Dove, E.L., P.G. Katona, Respiratory effects of brief baroreceptor stimuli in the anesthetized dog. *J. Appl. Physiol.*, 59:1258-1265 (1983).
4. Feldberg, W. and P.G. Guertzenstein, A vasodepressor effect of pentobarbitone sodium. *J. Physiol., Lond.* 224:83-103 (1972).
5. Granata, A.R., D.A. Ruggiero, D.H. Park, T.H. Joh, and D.J. Reis, Brain stem area with C1 epinephrine neurons mediates baroreflex vasodepressor responses. *Am. J. Physiol.*, 248(17):H547-H567 (1985).
6. Harvey, S.C., Hypnotics and sedatives. In A. Goodman Gilman, L.S. Goodman, T.W. Rall, F. Murad (Eds.), *The Pharmacological Basis of Therapeutics*, McMillan, New York, pp. 339-371 (1985).
7. McAllen, R.M., Location of neurones with cardiovascular and respiratory function, at the ventral surface of the cat's medulla. *Neuroscience*, 18(1):43-49 (1986).
8. Millhorn, D.E. and F.L. Eldridge, Role of the ventrolateral medulla in regulation of respiratory and cardiovascular systems. *J. Appl. Physiol.*, 61(4):1249-1263 (1986).
9. Sapru, H.N. and A.J. Krieger. Carotid and aortic chemoreceptor function in the rat. *J. Appl. Physiol.*, 42(3):344-348 (1977).
10. Sapru, H.N., E. Gonzaleez, and A.J. Krieger. Aortic nerve stimulation in the rat: Cardiovascular and respiratory responses. *Brain Res. Bull.*, 6:393-398 (1981).

Fig. 1 Effects of ADN stimulation on systemic arterial pressure (SAP), respiratory frequency (F), and respiratory flow (FLOW), before applying pentobarbital (PB) to the VMS (left panel), after applying PB (middle panel), and after cleaning the VMS with mock cerebrospinal fluid (mCSF) (right panel). Arrows on top of each panel indicate ADN stimulation on and off.

Fig. 2 Effects of pentobarbital application to the VMS. Top: (a) Baseline mean arterial pressure (MAP) and (b) baseline ventilation ($\dot{V}_e$). Bottom: Maximum responses to ADN stimulation, MAP (c) and $\dot{V}_e$ (d). 1 - Before applying PB to VMS, 2 - after applying PB to VMS, 3 - after cleaning VMS. n=5

Fig. 3 Effects of nicotine application to the VMS. Top: (a) Baseline mean arterial pressure (MAP) and (b) baseline ventilation ($\dot{V}_E$). Bottom: Maximum responses to ADN stimulation, MAP (c) and $\dot{V}_E$ (d). 1 - Before applying nicotine to VMS, 2 - after applying nicotine to VMS, 3 - after cleaning VMS. $n=5$

CHAPTER V¹

ROLE OF VENTRAL MEDULLARY SURFACE GABA_A RECEPTORS IN MEDIATING BAROREFLEX INHIBITION OF ARTERIAL PRESSURE AND VENTILATION IN THE RAT

INTRODUCTION

Arterial baroreceptor stimulation inhibits both arterial pressure and ventilation [4] (i.e, the "baro-pressure" and "baro-ventilatory" reflexes). Regions of the ventrolateral medulla are involved not only in vasomotor control and mediation of arterial baroreflexes, but also in respiratory control [8]. It has previously been shown that GABA_A receptors on neural structures under the intermediate area of the ventral medullary surface (VMS) of the cat affect both arterial pressure and ventilation as well as modulate the baro-pressure reflex [10,11]. Intracerebroventricular (i.c.v) injection of GABA or its α -agonists in the rat has also been shown to inhibit ventilation, primarily because of a decrease in tidal volume, and arterial pressure [3]. Although neural structures with GABA_A receptors near the VMS of the rat that affect the steady-state control of arterial pressure [5] may mediate the responses seen with i.c.v injections, the neural structures responsible for the ventilatory responses have not been determined. In a previous study we found that pentobarbital application to the

¹ This work was supported in part by NIH Program Project Grant HL25830.

VMS of the rat attenuated the baro-pressure reflex [4]. Since baseline blood pressure also decreased on pentobarbital application, the attenuation in the baro-pressure reflex could have been caused by a lower drive to the neural structures involved. The goal of this study was therefore to test the hypothesis that neural structures with GABA_A receptors adjacent to the VMS of the rat, in addition to playing a role in setting the level of arterial pressure, also affect the baseline control of ventilation and mediate the baro-pressure and baro-ventilatory reflexes. Unlike the study with pentobarbital, GABAergic blockade would result in an increased tonic activity.

METHODS

7 Sprague-Dawley rats weighing 417 ± 37 g (mean $\pm$ s.d.) were anesthetized with urethane (1.1 - 1.3 g/kg, i.p.). Rectal temperature was maintained at $36 \pm 1^\circ\text{C}$ with a heating pad and heating lamp. Cannulas in the femoral artery and femoral vein were used, respectively, for measuring arterial pressure and for infusions. A ventral cervical midline incision was made and the trachea, carotid arteries, vagi, aortic depressor nerves, and carotid sinus nerves were exposed. The trachea was cannulated and the animals spontaneously breathed O₂ enriched room air. A pneumotachometer was used to derive the respiratory flow. With the rats in supine position, the larynx and oesophagus were reflected and the underlying musculature excised to expose the occipital bone which was removed to form a window approximately 6-7 mm long and 5-6 mm wide. The dura was cut and reflected to expose the ventral medullary surface

(VMS).

As described in [4], the left or right aortic depressor nerve (ADN) was placed on Ag-AgCl bipolar stimulating electrodes and covered with petroleum jelly or mineral oil. The ADN was stimulated supramaximally with a train of square pulses of 3-5 volts, 0.2 msec duration, and 80-120 pulses per second repetition rate. The parameters for the pulse train were kept fixed for the entire experiment. To block GABA_A receptors on neural structures underlying the VMS, bicuculline (BIC) was applied bilaterally to the VMS (0.832 nmol each side in volumes of 1 μ l, diluted in mock cerebrospinal fluid, CSF or tris buffered saline, TBS using small pieces of gelfoam approximately 2 mm square. The gelfoam pieces were centered bilaterally, approximately 2 mm lateral to the basilar artery. Rostrocaudally the position of the center was approximately 3 mm rostral to the caudal edge of the occipital foramen. Arterial pressure and respiratory responses were obtained before and during the application of bicuculline, and again after cleaning the VMS with warm mock CSF or TBS.

The mean arterial pressure (MAP) and respiratory flow were sampled by a computer, and either off-line or on-line analysis was performed to determine the respiratory variables for each breath. Determination of inspiratory and expiratory times was used to obtain respiratory frequency F (in breaths/min), and integration of the respiratory flow yielded the tidal volume V_T (in ml) for each breath. Ventilation $\dot{V}_E$ (in ml/min) was calculated as the product of F and V_T . The data were smoothed

by a moving average of every 3-breaths. The baseline values were calculated by averaging this smoothed data over 5 breaths before ADN stimulation was started. Student's t-tests were used to determine if the decrease in MAP, $\dot{V}_E$, F, and V_T on ADN stimulation were significant. Analysis of variance (ANOVA) followed by the Tukey's test for multiple comparisons was used for comparisons of (1) the baseline levels of MAP, F, V_T , and $\dot{V}_E$ before, during, and after the application of bicuculline to the VMS, and (2) the maximum decreases in MAP, F, V_T , and $\dot{V}_E$ due to ADN stimulation during the same conditions. The effects were considered significant for $P \leq 0.05$. All data are given as mean $\pm$ S.E.M..

RESULTS

Fig. 1 illustrates the effect of ADN stimulation before and during bilateral BIC application on systemic arterial pressure (SAP), respiratory flow (FLOW), and tidal volume (V_T) in a rat. ADN stimulation reduced arterial pressure and ventilation both before and during bicuculline application to the VMS. Fig. 2 shows the baseline values of MAP, $\dot{V}_E$, F, and V_T and Figs. 3 and 4 show the absolute and percent change, respectively, in the four variables caused by ADN stimulation under the three conditions of pre-BIC application, with BIC on VMS, and after cleaning VMS. Table 1 gives the average baseline values for the four variables under the three conditions, while table 2 gives the average responses to ADN stimulation.

Considering all 7 animals (Table 1), application of BIC to the VMS caused an

increase in the baseline MAP from 101.0 ± 2.8 mmHg to 151.6 ± 4.4 mmHg, which was significantly greater than both the pre-BIC baseline and the baseline after washing the VMS. Washing the VMS with mock CSF or TBS reduced the baseline MAP back toward the pre-BIC values and there were no significant differences between the pre-BIC values and those after cleaning the VMS, the latter being 104.1 ± 4.3 mmHg. $\dot{V}_E$, F, and V_T were not significantly different on applying BIC to the VMS when compared to their pre-BIC and after cleaning the VMS values ($P = 0.6$, $P = 0.8$, and $P = 0.9$ for $\dot{V}_E$, F, and V_T respectively).

ADN stimulation before applying BIC to the VMS significantly decreased MAP by $34.9 \pm 4.5\%$, $\dot{V}_E$ by $32.8 \pm 6.5\%$, F by $13.3 \pm 3.8\%$ and V_T by $17.2 \pm 4.3\%$ (Table 2). After BIC application, ADN stimulation again significantly decreased all variables except F ($P = 0.1$). The decreases were $14.3 \pm 4.4\%$, $23.7 \pm 7.5\%$, $9.3 \pm 6.5\%$, and $11.1 \pm 4.7\%$ for MAP, $\dot{V}_E$, F, and V_T respectively. ANOVA indicated that on an absolute basis the reflex decrease in MAP on ADN stimulation when compared with the pre-BIC and post-BIC values had a tendency to be attenuated but the difference did not quite reach statistical significance ($P = 0.08$). However, it was significantly reduced when considered on a percentage basis ($P = 0.003$). No significant differences could be demonstrated in the reflex changes in the ventilatory variables whether considered on an absolute or percentage basis; the significance levels for the changes in absolute and % terms were $P = 0.4$ and $P = 0.5$ for $\dot{V}_E$, $P = 0.3$ and $P = 0.3$ for F, and $P = 0.8$ and $P = 0.7$ for V_T .

DISCUSSION

The aortic depressor nerve of the rat has been shown to contain purely baroreceptor afferents [9], hence ADN stimulation is considered to mimic aortic baroreceptor afferent stimulation. Application of bicuculline to the VMS raised the baseline MAP. This rise in MAP is even greater than the increase obtained by Keeler et al. [5]. The increase in baseline MAP suggests that GABAergic inhibitory inputs to the neural structures under the VMS may have been blocked since the area underlying the site of BIC application may corresponds to the pressor area in the rostral ventrolateral medulla (RVLM) of the rat which receives such an inhibition [12]. However, there were no significant changes in the baseline respiratory variables even though VT tended to increase and F tended to decrease. This is in contrast to the findings of Hedner et al. [3] who found a ventilatory depression on i.c.v injection of GABA and muscimol (a GABA_A agonist) in the rat, and those of Yamada et al. in the cat [11]. The latter found that bicuculline application to the Schlaefke area (this intermediate area of the VMS is considered to be an area for integration of respiratory responses; the pressor region of the RVLM lies under this) caused a significant increase in VT and hence $\dot{V}_E$, even though their increase in MAP of approximately 7.9% (Table 3 of [11]) was much lower than that observed by us (approximately 51%). Thus, since we did have a profound affect on baseline MAP and not $\dot{V}_E$ the results suggest that in the rat, unlike in the cat [11], neural structures near the VMS that are known to affect respiration [4,6,7] do not appear to have functionally

significant number of GABA_A receptors. The ventilatory affects observed by Hedner et al. are likely to be mediated by structures other than those near the VMS of the rat. In a previous study we had found that pentobarbital application to the VMS inhibited baseline ventilation [4]. However, barbiturates may act on all types of GABA receptors, and are thought to act at the ionophore portion of the receptor [2] while bicuculline only blocks the GABA_A receptor site. Pentobarbital can also act at other receptors, for example, at nicotinic receptors [1]. These differences in the action of the two drugs may explain the different observations with BIC and pentobarbital applications.

On applying BIC to the VMS the baro-pressure reflex showed a tendency to decrease. This decrease did not attain statistical significance when considered on an absolute basis. Considering on a percentage basis, the attenuation of the baro-pressure reflex was significant. The lack of baro-pressure reflex attenuation (in absolute values) could be because the dose of the drug used by us was not sufficient. Since BIC application increased the baseline blood pressure considerably, the lack of significant attenuation is more likely due to the neural structures that mediate the baro-pressure reflex being located deeper and the BIC applied to the VMS not diffusing deep enough to access them. The results suggest the existence of inhibitory GABAergic inputs to the sympathetic neurons from neural structures that are not part of the baro-pressure pathway.

The baro-ventilatory reflex was also not attenuated significantly, suggesting that

neural structures near the VMS are unlikely to be part of the baro-ventilatory pathway. These results also confirm a previous study in which pentobarbital or nicotine application on the VMS did not alter the baro-ventilatory reflex [4].

In conclusion, neural structures near the VMS that have GABA_A receptors affect vasomotor tone but not ventilation. Additionally, while these structures may participate in the baro-pressure reflex, they are not likely to play a role in the baroreflex mediated control of ventilation.

REFERENCES

1. Collinridge, G.L. and R.A.J. Lester. Excitatory amino acid receptors in the vertebrate central nervous system. *Pharmacol. Rev.*, 40(2):143-210 (1989).
2. Dailey, J.W. Sedative-hypnotic and anxiolytic agents. In: *Modern Pharmacology*. Charles R. Craig and Robert E. Stitzel (eds.) Little, Brown and Co., Boston. Chp. 32, p. 440-452 (1990).
3. Hedner, J., T. Hedner, J. Jonason, and D. Lundberg, GABA-ergic mechanisms in central respiratory control in the anesthetized rat. *Naun. Schm. Arch. Pharmac.*, 317:315-320 (1981).
4. Jung, R, E.N. Bruce, and P.G. Katona, Tonic and baroreflex effects on arterial pressure and ventilation of pentobarbital and nicotine on the rat ventral medullary surface. *Brain Research*, 485:399-402 (1989).
5. Keeler, J.R., C.W. Shults, T.N. Chase, and C.J. Helke. The ventral surface of the medulla in the rat: Pharmacological and autoradiographic localization of GABA-induced cardiovascular effects, *Brain Research*, 297:217-224 (1984).
6. Martin, R.L. and J.D. Sinclair. Kainic acid on the rat ventral medullary surface depresses hypoxic and hypercapnic ventilatory responses, *Resp. Physiol.*, 80:55-70, (1990).
7. Malcolm, J.L., I.H. Sarelius, and J.D. Sinclair. The respiratory role of the ventral surface of the medulla studied in the anesthetized rat, *J. Physiol.*, 307:503-515 (1980).
8. Millhorn, D.E. and F.L. Eldridge, Role of the ventrolateral medulla in regulation of respiratory and cardiovascular systems. *J. Appl. Physiol.*, 61(4):1249-1263 (1986).
9. Sapru, H.N. and A.J. Krieger. Carotid and aortic chemoreceptor function in the rat. *J. Appl. Physiol.*, 42(3):344-348 (1977).
10. Yamada, K.A., R.M. McAllen, and A.D. Loewy. GABA antagonists applied to the ventral surface of the medulla oblongata block the baroreceptor reflex. *Brain Research*, 297:175-180 (1984).
11. Yamada, K.A., W.P. Norman, P. Hamosh, and R.A. Gillis. Medullary ventral

surface GABA receptors affect respiratory and cardiovascular function. *Brain Research*, 248:71-78 (1982).

12. Willette, R.N., A.J. Kriegger, P.P. Barcas, and H.N. Sapru. Medullary γ -aminobutyric acid (GABA) receptors and the regulation of blood pressure in the rat. *J. Pharmacol. Exp. Therap.*, 226(3):893-899 (1983b).

Table 1 Effects of bicuculline (BIC) application to the ventral medullary surface of 7 rats on baseline mean arterial pressure (MAP), ventilation ($\dot{V}_E$), respiratory frequency (F), and tidal volume (V_T).

	BEFORE BIC	WITH BIC	AFTER BIC
MAP (mmHg)	101.0 $\pm$ 2.8	151.6 $\pm$ 4.4*	104.1 $\pm$ 4.3
$\dot{V}_E$ (ml/min)	212.5 $\pm$ 13.9	224.7 $\pm$ 18.1	235.8 $\pm$ 13.9
F (b/min)	123.5 $\pm$ 14.7	114.5 $\pm$ 12.0	128.4 $\pm$ 13.7
V_T (ml)	1.9 $\pm$ 0.2	2.0 $\pm$ 0.3	2.0 $\pm$ 0.3

Values are mean $\pm$ standard error of the mean.

* indicates significantly different from the other conditions at $P \leq 0.05$. ANOVA followed by Tukey's test was used for comparisons.

Table 2 Effects of bicuculline (BIC) application to the ventral medullary surface of 7 rats on baroreflex responses (Δ MAP, $\Delta\dot{V}_E$, ΔF , and ΔV_T).

	BEFORE BIC	WITH BIC	AFTER BIC
Δ MAP (mmHg)	34.7 $\pm$ 4.2*	21.1 $\pm$ 6.4*	36.8 $\pm$ 4.3*
$\Delta\dot{V}_E$ (ml/min)	70.3 $\pm$ 15.4*	52.0 $\pm$ 15.2*	82.3 $\pm$ 19.0*
ΔF (b/min)	16.7 $\pm$ 6.0*	9.2 $\pm$ 7.3	23.3 $\pm$ 3.4*
ΔV_T (ml)	0.4 $\pm$ 0.1*	0.3 $\pm$ 0.1*	0.2 $\pm$ 0.1

Values are mean $\pm$ standard error of the mean.

* indicates significant Δ at $P \leq 0.05$.

Fig. 1 Effects of ADN stimulation on systemic arterial pressure (SAP), respiratory flow (FLOW), and tidal volume (VT) before applying bicuculline (BIC) to the VMS (left panel) and with BIC on the VMS (right panel). Arrows on top of each panel indicate ADN stimulation on and off.

Fig. 2 Effects of BIC application to the VMS. Top: (a) Baseline mean arterial pressure (MAP) and (b) baseline ventilation (VE). Bottom: (c) Baseline frequency (F) and (d) baseline tidal volume (VT). 1 - Before applying bicuculline to VMS, 2 - after applying bicuculline to VMS, 3 - after cleaning VMS. $n = 7$

Fig. 3 Maximum responses to ADN stimulation (absolute change). Top: (a) Delta MAP (Δ MAP) and (b) Delta VE (Δ VE). Bottom: (c) Delta F (Δ F) and (d) Delta VT (Δ VT). 1 - Before applying bicuculline to VMS, 2 - after applying bicuculline to VMS, 3 - after cleaning VMS. $n = 7$

Fig. 4 Maximum responses to ADN stimulation (percentage change). Top: (a) Delta MAP (Δ MAP) and (b) Delta VE (Δ VE). Bottom: (c) Delta F (Δ F) and (d) Delta VT (Δ VT). 1 - Before applying bicuculline to VMS, 2 - after applying bicuculline to VMS, 3 - after cleaning VMS. $n = 7$

CHAPTER VI¹

CARDIO-RESPIRATORY RESPONSES TO GLUTAMATERGIC ANTAGONISTS IN THE CAUDAL VENTROLATERAL MEDULLA OF RATS

INTRODUCTION

Anatomical, electrophysiological, and functional data have shown that neurons in the caudal ventrolateral medulla (CVLM) of the rat have a reciprocal inhibitory connection with the sympathetic neurons of the rostral ventrolateral medulla which control arterial pressure [1,4,6,32]. The CVLM neural structures may also be obligatory for mediating the baroreflex inhibition of blood pressure [2,13,19,29,31] and the vagal baroreflexes [15]. Neurons in this area have reciprocal connections with the hypothalamus and supraoptic nucleus [6] thereby providing a pathway for neurohumoral autonomic control. Additionally, anatomical and electrophysiological studies indicate that within the CVLM lie respiratory neurons [9,10,27,28]. It has been suggested that cells in this region can affect both cardiovascular and respiratory control [5]. Therefore, the neural structures in the CVLM could play an important role in integrating the cardiovascular and respiratory systems.

Relatively little is known about the neurotransmitters utilized by neural circuits that mediate the control of cardiovascular and respiratory function [6,8,23,31]. The excitatory connection from the NTS to the CVLM that affects arterial pressure was

¹ This work was supported in part by NIH Grant HL-25830

shown to be glutamatergic [30]. Since there exist neurons with respiration related activity in the CVLM, endogenous glutamate may act on them to alter respiratory function.

Therefore, our goal was to test the hypothesis that (a) CVLM neurons play a role in the baroreflex control of arterial pressure and ventilation, and b) the control of arterial pressure and ventilation as well as the baroreflexes are mediated by *N*-methyl-D-aspartate (NMDA) and non-NMDA (kainate/quisqualate) excitatory amino acid receptors on these neurons.

METHODS

General Procedure

Male Sprague-Dawley rats were anesthetized with urethane (1.2 g/kg i.p., additional doses as necessary). Rectal temperature was maintained at 37 ± 0.5 C using a heating pad. Cannulas in the femoral artery and femoral vein were respectively used for measuring systemic arterial pressure (SAP) and for infusions. Following a ventral cervical midline incision, the trachea and aortic depressor nerves were exposed. The trachea was cannulated and the animals either spontaneously breathed O₂-enriched room air or, if necessary, were artificially ventilated (Harvard Apparatus, 60-70 strokes/min, 1-1.2 ml/100 g body weight). A pneumotachometer was used to measure the respiratory flow. A biased flow system prevented

rebreathing in spontaneously breathing rats. In some animals changes in end-tidal CO₂ were measured by sampling the expiratory flow with a capnograph (Gould).

Procedure to obtain baroreflexes

The right or left aortic depressor nerve (ADN) was placed on Ag-AgCl bipolar stimulating electrodes and covered with petroleum jelly. It was then supramaximally stimulated using a train of square pulses at 5 volts, 2 ms duration, and 100 pulses per second, to obtain a reflex transient decrease in systemic arterial pressure (baro-pressure reflex). These parameters for supramaximal stimulus were determined previously [17]. The baro-pressure reflex (BPR = delta mean arterial pressure in mmHg) was determined as the difference between the control MAP before ADN stimulation and the minimum MAP on ADN stimulation.

Ventral medullary preparation and microinjection technique

As previously described [17], with the rats in the supine position in a stereotaxic frame (Trentwell), the larynx and oesophagus were reflected and the underlying musculature excised to expose the occipital bone. The bone was then removed to form a window approximately 6-7 mm long and 5-6 mm wide. The dura was cut and reflected to expose the ventral medullary surface and the XIIth hypoglossal rootlets.

Drugs were dissolved in tris buffered saline (TBS) and pH adjusted to 7.3-7.4

with 1 N NaOH. A single barrel glass micropipette (AM Systems) pulled to a long shank with a tip of 20-50 μm external diameter filled with the drug solution was connected via polyethylene tubing to a 3 cc syringe. Air pressure was used for injection; the movement of the meniscus in the pipette was monitored under a microscope equipped with a graduated reticule to deliver 30-32 nl or 50 nl volumes. Based on previous landmarks for the caudal ventrolateral depressor area (VLDA) as indicated by Willette et al. [32], Gordon [13], and the coordinates in the stereotaxic atlas by Paxinos and Watson [26], microinjections were made at the second hypoglossal rootlet in the CVLM. The depth (approximately 0.9 mm) and laterality (approx. 1.7 - 2 mm) were measured from the ventral medullary surface (VMS) and basilar artery, respectively. The pipette was withdrawn soon after the microinjection. All sites in the CVLM were functionally verified at the end of the protocol to be in the caudal depressor area by microinjection of 2 nmol of L-glutamate and observing the fall in blood pressure. They were also marked by dye microinjection as explained later. The same pipette was used for all microinjections. Before replacing the drug in the pipette with L-glutamate or dye (for marking the site), it was repeatedly suction cleaned with distilled water. A visible depression caused in the ventral medullary surface by the first microinjection and the stereotaxic coordinates used were utilized for subsequent microinjections. Microinjection of vehicle (adjusted to the same pH as the dissolved drug) did not cause any observable responses.

Histological verification

The sites of drug delivery were marked by microinjection of corresponding volumes of indelible Sharpie marker dye dissolved in absolute alcohol [14] or of 25% india ink in TBS. The brains were removed and stored in 10% phosphate buffered formalin. Serial coronal sections (30 μ) were obtained using a vibratome. Sections that contained the dye were consequently stained with hematoxylin and eosin, and then mounted. Using a light microscope the site of microinjection was determined and the neural structures surrounding it identified by comparing them to a standard rat atlas [26]. Finally, the sites were marked on outline sketches of the projected sections.

Data acquisition and analysis

The SAP, mean arterial pressure (MAP, obtained by low pass filtering the SAP at 0.8 Hz), respiratory flow and tidal volume (VT, integrated respiratory flow reset to zero at respiratory phase transitions) were obtained and displayed on a strip-chart recorder (Gould). The waveforms were also sampled on-line at 350 Hz each using a DT-2801A A/D board (Data Translation) and recorded real time on an IBM-AT compatible personal computer. Further analysis was done off-line. A computer program was used to determine inspiratory time and expiratory time for each breath, and the corresponding MAP. The respiratory rate, F in breaths/min, inspiratory tidal volume, VT, in ml (integral of inspiratory flow), and ventilation, $\dot{V}_E$, in ml/min (product of F and VT) were also calculated on a breath by breath basis.

The above parameters were averaged for six breaths before drug microinjection to obtain the control levels. After the drug microinjection, six breaths were averaged at every ten second interval to obtain a time course of the MAP, V_T , F , and $\dot{V}_E$ responses. In experiments where apnea occurred (see results below), final values were calculated as the averages of the variables for the last 6 breaths before respiratory flow ceased.

Statistical analysis

Paired comparisons were made using Student's t-tests where appropriate. Differences were considered statistically significant at $P \leq 0.05$. Values reported are mean $\pm$ standard error of the mean (s.e.m.) unless specified otherwise.

PROTOCOLS

Three main sets of experiments were performed in which different glutamate blockers were microinjected. In the first set, control baroreflexes, steady-state MAP, and ventilation were obtained and then the broad-band glutamatergic antagonist kynurenic acid (KYN) microinjected in the right or left VLDA in a random order. Either of 2 doses of KYN (5 nmol in 50 nl or 1.58 nmol in 31 nl) was used. At the concentrations used kynurenic acid acts at the NMDA, kainate and quisqualate receptor sites [7]. After the responses to this unilateral microinjection had stabilized, the contralateral side was microinjected using the same dose. The baro-pressure reflex

was again obtained. In some rats KYN (25 nmol in 0.5 μ l) was bilaterally applied to the VMS above the VLDA using 1 mm² gelfoam pledgets before microinjection was attempted. In some other rats low dose KYN was microinjected approximately 2.8 mm rostral to the 2nd hypoglossal rootlet. Both the surface applications and rostral microinjections were done to establish the specificity of the neural structures in the region of the VLDA for the responses observed, and, when done, preceded the caudal microinjections by at least half an hour. Since kynurenic acid blocked ventilation (see RESULTS), the animals had to be ventilated as soon as respiratory arrest occurred.

Protocol 2 was similar to protocol 1 but tested whether NMDA receptors mediate the tonic ventilatory as well as tonic and baroreflex blood pressure responses obtained on KYN microinjection. For this, the specific NMDA antagonist 2-amino-5-phosphonovaleric acid (2APV) [7] rather than KYN was microinjected in the VLDA. The dose used was 2.7 nmol in 31 nl which has been shown to be sufficient for blocking the baroreflexes [13].

Protocol 3 was similar to protocols 1 and 2, except that this protocol tested if non-NMDA receptors mediate the tonic ventilatory, tonic blood pressure, and baroreflex responses obtained on KYN microinjections. Hence, 0.257 nmol in 31 nl of the non-NMDA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) was used as the blocking agent. In low micromolar doses CNQX acts at kainate and quisqualate receptor sites and is a very potent specific non-NMDA antagonist [16,33]. The dose chosen was based on a few preliminary experiments and a similar

experiment in which bilateral microinjection of 100 pmol of DNQX, a non-NMDA antagonist slightly less potent than CNQX [16], into the nucleus tractus solitarius of anesthetized rats was sufficient to attenuate the baro-pressure reflex [20].

Only one excitatory amino acid (EAA) blocker at a single dose was used in each rat. In all three protocols, an attempt was made in some animals to stimulate spontaneous breathing during ventilatory block by intermittent ventilation with 5% CO₂. The mean increase in end-tidal CO₂ (1-2%) was verified by sampling the expiratory flow with a Gould capnograph.

RESULTS

Effects of broad-band antagonist kynurenic acid

Caudal depressor area microinjections

Figure 1 shows a typical response of respiratory flow and arterial pressure to unilateral high dose (5 nmol) microinjection of kynurenic acid in the VLDA. Fig. 2 shows the time course of the responses for MAP, F, VT, and $\dot{V}_E$ for each of the 4 rats. Within 10 seconds after the last values shown, respiratory flow could no longer be detected. On microinjection there was a short apnea for 7.8 ± 3 seconds (mean $\pm$ standard deviation (s.d.)). The MAP increased immediately and no change in heart-rate (as noted from the pressure pulses) was observed. Following the apnea, a

rebound recovery of ventilation occurred, but $\dot{V}_E$ slowly decayed till breathing ceased after 69 ± 27 seconds (mean $\pm$ s.d.) and the animal had to be artificially ventilated. The decrease in $\dot{V}_E$ was caused by a fall in V_T ; respiratory rate increased from 126 ± 24 to 264 ± 90 breaths/min as V_T decreased. When the arterial pressure had stabilized, the same dose of KYN was microinjected into the contralateral VLDA which resulted in an immediate further rise in MAP. A typical response to ADN stimulation before and after bilateral microinjections of KYN (5 nmol on each side) is shown in Figure 3. The inhibition of MAP on ADN stimulation observed before microinjecting KYN was eliminated or converted to an excitation after bilateral microinjections. On average, as indicated in Table 1, the baseline MAP level after bilateral microinjections was significantly greater than before drug microinjections by 77 ± 23 mmHg. The change in BPR (-29 ± 4 mmHg before and $+20 \pm 12$ mmHg after) was also significant. Approximately 15 to 20 min after the second microinjection, the baseline MAP started decreasing to the original baseline that existed before KYN microinjections. After approximately another 1-1.25 hours spontaneous breathing as well and BPR recovered, though not always to the control levels.

Microinjections were also made with the lower dose (1.58 nmol). Low dose caudal microinjections of KYN caused responses similar to those obtained with the high dose. A typical response is shown in Figure 4; the time profiles presented in the right panel of Figure 5. After unilateral microinjection into the VLDA of 4 rats, MAP

increased. Although the short apnea observed with the high dose was absent and the response of $\dot{V}_E$ was slower, breathing again ceased 143 ± 85 sec (mean $\pm$ s.d.) after injection (n=4), and the animals had to be artificially ventilated. The fall in $\dot{V}_E$ was again caused by a diminished V_T ; F increased from 138 ± 18 to 300 ± 46 breaths/min. After bilateral microinjections the baseline MAP increased significantly from the pre-drug level by 88 ± 13 mmHg. BPR was abolished, and converted from an inhibitory to an excitatory response (-31 ± 12 mmHg before and $+12 \pm 2$ mmHg after bilateral KYN). Approximately 8-12 minutes after the second microinjection baseline MAP started decreasing towards control, and was followed only after another 1-1.25 hours by recovery of BPR and spontaneous breathing.

Four animals (1 high dose and 3 low dose KYN microinjections) were ventilated with 5% CO₂ for 2-4 minutes during apnea in an attempt to restore breathing through a chemoexcitatory stimulus. Although the arterial pressure increased, the animals made no respiratory effort.

Rostral microinjections and VMS application

As explained in the protocol section, microinjections of low dose were also made at different distances rostral to the corresponding VLDA sites at approximately the same laterality and depth. As shown in the time profiles of the responses in the left panel of Fig. 5, microinjections at 7 rostral sites in 4 rats (only one injection per medullary half) yielded no significant changes in MAP, $\dot{V}_E$, F, or V_T .

In 4 rats 25 nmol KYN in 0.5 μ l was applied on the ventral medullary surface bilaterally above the VLDA. There were no discernible changes in MAP or respiratory flow, suggesting that neural structures near the ventral medullary surface were not responsible for mediating the effects seen by the microinjections.

Effects of NMDA antagonist 2APV

To determine if NMDA receptors were the glutamatergic sites that were blocked by KYN in the previous experiments, the cardio-respiratory responses were also determined after NMDA receptor blockade. For this, after obtaining the control values for MAP, F, VT, $\dot{V}_E$ and the baro-pressure reflex, 2.7 nmol of 2APV, a specific NMDA antagonist, was microinjected unilaterally into the VLDA. Fig. 6 shows a typical arterial pressure and respiratory flow response and the right panel of Fig. 7 gives the time profiles of the MAP, $\dot{V}_E$, F, and VT responses. In 5 rats, unilateral 2APV microinjection in the VLDA caused an increase in MAP, and decrease in respiratory flow until ventilation ceased in 103 ± 71 seconds (mean $\pm$ s.d.) and the animals had to be ventilated artificially. The decrease in $\dot{V}_E$ was caused mainly by a decrease in VT even though F also had a tendency to decrease from 101 ± 6 breaths/min at control to 86 ± 13 breaths/min (N.S). After bilateral microinjections in the VLDA, the MAP again rose, and as summarized in Table 1, this increase (43 ± 13 mmHg) was significant. BPR was significantly altered by conversion from an inhibitory reflex before (-37 ± 5 mmHg) to an excitatory reflex ($+7 \pm 3$ mmHg) after

the bilateral microinjections. The baseline MAP started recovering in 8-20 min after the second microinjection, and after another 1 - 1.25 hours ventilation and the baro-pressure reflex recovered in 4 out of the 5 rats. Ventilation of all 5 rats with 5% CO₂ during the apnea did not stimulate respiration even though the blood pressure increased. In 2 rats (3 sites), microinjections of 2.7 nmol 2APV were made in the rostral medulla before microinjections in the VLDA were made. As shown in the time profiles in the left panel of Fig. 7 there were no significant affects on MAP, $\dot{V}_E$, F, and VT.

Effects of non-NMDA antagonist CNQX

To determine if the responses due to KYN microinjection in the VLDA were mediated via non-NMDA receptors, the antagonist CNQX, a specific potent non-NMDA antagonist was microinjected into the VLDA. The response of arterial pressure and respiratory flow to a unilateral microinjection in a rat is shown in Fig. 8, and the time profiles for the MAP, $\dot{V}_E$, F, and VT responses in 5 rats are shown in the right panel of Fig. 9. Unilateral microinjection of 0.257 nmol of CNQX caused immediate respiratory arrest in 4 rats. Of these, in 2 rats the respiratory arrest was sustained, but 2 showed a rebound increase in ventilation. However, in both these rats and the fifth rat that did not show an immediate respiratory arrest, ventilation decayed to zero flow in 23 ± 11 seconds (mean $\pm$ s.d.). Concomitantly, the F for these 3 rats increased from 199 ± 37 breaths/min to 332 ± 77 breaths/min. For all 5 rats MAP

failed to increase on unilateral microinjection. After bilateral microinjections of CNQX the MAP still did not change, and the net change in baseline MAP (-1.0 ± 10 mmHg) was not significant. However, the BPR which was -38 ± 9 mmHg before CNQX microinjections was significantly attenuated and a reflex response of $+5 \pm 5$ mmHg was obtained after bilateral CNQX.

After 1-1.5 hours breathing recovered in 4 rats. During the respiratory arrest, ventilation with 5% CO₂ caused an increase in arterial pressure but did not stimulate breathing. In 4 rats CNQX was also microinjected at 8 sites (one site in each medullary half) in the rostral medulla. As shown in the left panel of Fig. 9 there were no significant effects on MAP, F, VT, or $\dot{V}_E$.

Histological verification

Figure 10 shows the histological site locations for the microinjections in the caudal and rostral ventrolateral medulla. In the caudal medulla, the KYN microinjection sites were 0.96 ± 0.19 mm (mean $\pm$ s.d.) under the VMS and 1.77 ± 0.25 mm lateral to the midline. The 2APV sites were 1.01 ± 0.11 mm (mean $\pm$ s.d.) under the VMS and 1.82 ± 0.23 mm lateral to the midline, and the CNQX microinjection sites were 0.96 ± 0.19 mm under the VMS and 1.81 ± 0.18 mm lateral to the midline. Kynurenic acid was microinjected 2.8 ± 0.2 mm rostral to the caudal sites, while 2APV and CNQX microinjections were 2.7 ± 0.1 and 2.4 ± 0.2 mm rostral to the corresponding caudal sites respectively.

The structures surrounding the caudal sites are best represented by plates 73 through 75 of the rat atlas by Paxinos and Watson [26], while those surrounding the rostral sites are best represented by plates 62 through 65. The majority of the caudal sites lay in the CVL between the nu. retroambiguus and the lateral reticular nucleus, while the majority of the rostral sites lay in the facial nucleus. The coronal section identifying the obex level in the atlas of the wistar rat brain by Paxinos and Watson (plate 75) does not coincide with the drawings of coronal sections identifying the obex level in sprague-dawley rats in other studies (see Fig. 1 of [5] and Fig. 2 of [15]). Therefore, since we ourselves only used the 2nd hypoglossal rootlet as the identifying site for microinjection into the CVLM we have not reported the rostro-caudal distance with respect to the obex. All caudal sites however, lie in the ventrolateral depressor area of the medulla as identified by the neural structures surrounding them and the functional response to L-glutamate.

DISCUSSION

Effects on arterial pressure and its baroreflex control

The results of this study show that neural structures in the caudal ventrolateral medulla of the rat affect both vasomotor tone and the aortic baroreflex control of blood pressure. The receptors that mediate the effect on vasomotor tone and hence the steady-state baseline MAP are likely of the NMDA type, while receptors

mediating the transient inhibition of blood pressure during aortic baroreceptor stimulation being both NMDA and non-NMDA type.

Previous studies have suggested that excitatory amino acid receptor sites on VLDA neurons are responsible for their excitation [13,15,18,29,31], and that the VLDA neurons, in turn, inhibit the sympathetic neurons in the rostral medulla [1,30,31], thus providing a tonic inhibition of basal blood pressure. The excitatory amino acid receptor sites in the VLDA have been suggested to be mainly of the NMDA type since microinjections of the NMDA blocker 2-APV in the VLDA by itself caused an increase in the basal blood pressure [18,29]. Our results support these conclusions, since we too obtained an increase in basal blood pressure on 2APV microinjection. Additionally, our results indicate that non-NMDA sites are probably not responsible for the action of the endogenous excitatory amino-acids on CVLM neurons that inhibit the rostral sympathetic neurons, since blockade with CNQX, a specific non-NMDA blocker, did not cause a rise in MAP.

The rise in the basal levels of blood pressure on 2APV and KYN microinjections is not secondary to reflex chemoexcitation due to ventilatory inhibition because a) the response on unilateral microinjection of 2APV or low dose KYN occurred prior to significant inhibition of ventilation, b) microinjection of 2APV and both doses of KYN in the contralateral half of the CVLM caused an additional increase in MAP while the animal was being artificially ventilated (thus keeping CO₂ constant), and c) in rats where CNQX was microinjected MAP did not rise in spite

of the ventilatory inhibition (which could have caused chemoexcitation).

The VLDA in the rat also appears to be an obligatory synapse in the baro-pressure reflex. Several previous studies, using NTS stimulation, aortic depressor nerve stimulation, and intravenous phenylephrine injection have shown that blockade of neurons in the CVLM causes attenuation or abolishment of the baroreflex inhibition of blood pressure [2,13,15,29,30]. The receptor sites in the VLDA have been suggested to be of the NMDA type [13,18,29]. Though NMDA receptor sites are indeed involved in the mediation of the baroreflexes, our results indicate that non-NMDA receptor sites are also capable of mediating the reflexes. After bilateral blockade of NMDA or non-NMDA sites in the VLDA the baro-pressure reflex was paradoxically converted from an inhibitory to an excitatory reflex. This paradoxical conversion confirms previous findings that suggest an excitatory pathway from the NTS to the RVLM sympathetic neurons [30] that is unmasked when the activity of the caudal depressor neurons is blocked. The net baroreflex effect on arterial pressure after either NMDA or non-NMDA block in the CVLM could thus result from the balance between the direct excitatory input from the NTS to the rostral pressor neurons and the indirect inhibitory input from the caudal depressor neurons.

The effects of excitatory amino acid receptor blockade on arterial pressure and the transient inhibition of arterial pressure on ADN stimulation, the baro-pressure reflex, were different. On blocking the EAA receptors in the VLDA with kynurenic acid, the basal blood pressure increased and the baro-pressure reflex was abolished.

However, the baseline blood pressure recovered before the baro-pressure reflex. Additionally, only NMDA receptor block increased the tonic blood pressure. These results suggest two possibilities, 1) that there exists a population of neurons with NMDA receptor sites that directly or indirectly inhibits the rostral sympathetic neurons independent of baroreceptor mechanisms, or 2) that the neural population in the VLDA that mediates the baro-pressure inhibition of the sympathetic neurons receives a tonic input drive mediated by NMDA receptor sites. Since the recovery of baseline blood pressure occurs before the baro-pressure reflex, this second possibility would require secondary non-baroreceptor mediated mechanisms that speed the recovery of baseline arterial pressure back to normal.

It is unlikely that our responses are due to diffusion of the drugs to other nearby neural structures that affect cardiovascular control. VMS applications of KYN, and microinjections of the blockers in various sites rostral to the VLDA sites did not cause significant changes in the blood pressure. This is consistent with the demonstration that a 50 nl drug or india ink microinjection diffuses within a sphere not greater than 700 μm in diameter [30].

Effects on ventilation

The results show that unilateral microinjections of glutamatergic blockers in the VLDA result in a profound inhibition of ventilation resulting in respiratory arrest. The cessation of sustained ventilation is mainly due to a decrease in the tidal volume

because the respiratory rate increased on KYN microinjection. This rise in rate is unlikely to be solely due to reflex chemoreceptor-mediated ventilatory excitation since in the case of 2APV microinjection a similar decrease in VT occurred but respiratory frequency did not increase. The respiratory arrest observed is also unlikely to be mediated by reflex baroreceptor inhibition because, in the case of CNQX blockade, the apneusis occurred in spite of the absence of a rise in MAP.

Studies in in vitro preparations of the isolated brainstem-spinal cord of the neonatal rat have suggested that there exists a pacemaker for rhythm generator separate from the neural structures generating the respiratory pattern [3,11]. In this preparation unilateral lesion of the caudal ventrolateral medulla at the medial hypoglossal rootlets abolished C4 activity but did not abolish the respiratory rhythm in more rostral pre-inspiratory neurons [3,23]. Similarly, a partial rostro caudal midline lesion at the hypoglossal rootlet level decreased the C4 activity but did not affect the pre-inspiratory neuron firing [25]. Our microinjections of EAA antagonists are similarly located under the medial hypoglossal rootlets between the lateral reticular nucleus and the nucleus ambiguus. Assuming the "pacemaker theory" for respiratory rhythm generation, the ventilatory profiles observed by us suggest that the respiratory arrest observed is due to block of an input to the pattern generator, rather than on rhythm generation.

Since unilateral blockade resulted in cessation of ventilation, important reciprocal functional connections must exist between the two medullary halves such

that reduction in the activity of one half of the medulla is sufficient to prevent functional ventilation. Similar results have been shown by unilateral cooling of the ventral medullary surface of the rat at the hypoglossal rootlets [22]. Several recent studies in the rat have shown that the CVLM region near the obex, called the rostral ventral respiratory group (rVRG), has a dense population of bulbospinal neurons with both inspiratory and expiratory patterns of discharge that have contralateral and ipsilateral monosynaptic projections to the phrenic motoneurons [9,26], the contralateral projections being slightly greater than the ipsilateral projections. Similarly, propriobulbar neurons of this region have dense reciprocal connections with the corresponding contralateral medullary half, as well as projection to and from other regions of the medulla and pons [10]. The profound effects of unilateral microinjections could be caused by these dense functional reciprocal connections between the two medullary halves.

 Injections of excitatory amino-acids in the rVRG and under the ventral medullary surface of the cat [12,19,21], and in the caudal ventrolateral medulla of the rat [5] have indicated that receptor sites on neurons that affect respiration are glutamatergic. Our results show that amino-acids in the caudal ventrolateral depressor area of the rat provide a tonic excitatory input for ventilation mediated via both NMDA and non-NMDA receptors. Our observation that ventilation ceases after KYN administration is in partial agreement with the results obtained by McCrimmon et al. in the cat [21]. Blockade of endogenously released excitatory amino-acids by KYN

in their study caused a depression in the peak activity of the phrenic nerve but did not cause apnea [21]. The disparity in our results in the rat and those observed by Mc Crimmon et al. in the cat may be because 1) our microinjection volumes were larger than those in the cat, thereby permitting blockade of greater number of receptor sites, 2) our microinjections sites were caudal to the relative sites in the cat, and 3) the area we have investigated is of greater importance in maintaining sustained ventilation in the rat than in the cat.

Though microinjections of excitatory amino acids in the VLDA affected both arterial pressure and ventilation, the effects could sometimes be separated. For example, non-NMDA blockade by CNQX microinjections did not affect baseline blood pressure but caused respiratory arrest. Also, the initial increase in baseline arterial pressure on kynurenic acid microinjections in the VLDA returned to control much faster than recovery of ventilation. These results suggest that the neural populations affecting control of arterial pressure are different from those that affect ventilation even though they are co-mingled.

In conclusion : (a) Confirming previous studies, the caudal ventrolateral depressor area in the rat appears obligatory for mediating the baroreflex inhibition of arterial pressure and these neural structures inhibit the sympathetic neurons providing the vasomotor tone; (b) Even unilateral block of neural structures in the VLDA resulted in ventilatory cessation, therefore they are essential for sustaining ventilation; (c) Since unilateral blockade abolishes ventilation, reciprocal excitatory coupling must

exist between the right and left caudal medulla; (d) Only NMDA receptors mediate control of baseline blood pressure while both NMDA and non-NMDA receptors mediate the baro-pressure reflex; (e) Both NMDA and non-NMDA receptor activation is necessary to sustain ventilation. The results hence suggest that the caudal ventrolateral medulla is an important area for mediating cardio-respiratory control.

REFERENCES

1. Agarwal, S.K., A.J. Gelsema, and F.R. Calaresu. Neurons in rostral VLM are inhibited by chemical stimulation of caudal VLM in rats. *Am. J. Physiol.*, 257: R265-R270 (1989).
2. Agarwal, S.K., A.J. Gelsema, and F.R. Calaresu. Inhibition of rostral VLM by baroreceptor activation is relayed through caudal VLM. *Am. J. Physiol.*, 258:R1271-R1278 (1990).
3. Arata, A., H. Onimaru, and I. Homma. Respiration-related neurons in the ventral medulla of newborn rats in vitro. *Br. Res. Bull.*, 24:599-604 (1990).
4. Barman, S.M. and G.L. Gebber. Lateral tegmental field neurons of cat medulla: A source of basal activity of ventrolateral medullospinal sympathoexcitatory neurons. *J. Physiol.*, 57(5):1410-1424 (1987).
5. Bonham, A.C. and I. Jeske. Cardiorespiratory effects of DL-homocysteic acid in caudal ventrolateral medulla. *Am. J. Physiol.* 256:H688-H696 (1989).
6. Ciriello, J., M.M. Caverson, and C. Polosa. Function of the ventrolateral medulla in control of the circulation. *Brain Res. Rev.*, 11:359-391 (1986).
7. Collinridge, G.L. and R.A.J. Lester. Excitatory amino acid receptors in the vertebrate central nervous system. *Pharmacol. Rev.*, 40(2):143-210 (1989).
8. Eldridge, F.L. and D.E. Millhorn. Central regulation of respiration by endogenous neurotransmitters and neuromodulators. *Ann. Rev. Physiol.*, 43:121-135 (1981).
9. Ellenberger, H.H. and J.L. Feldman. Monosynaptic transmission of respiratory drive to phrenic motoneurons from brainstem bulbospinal neurons in the rat. *J. Comp. Neurol.*, 269:47-57 (1988).
10. Ellenberger, H.H. and J.L. Feldman. Brainstem connections of the rostral ventral respiratory group of the rat. *Brain Research*, 513:35-42 (1990).
11. Feldman, J.L. and J.C. Smith. Cellular mechanisms underlying modulation of breathing pattern in mammals. *Ann. N.Y. Acad. Sc.*, 563:114-130 (1989).
12. Gatti, P.J., W.P. Norman, A.M. Taeira DaSilva, and R.A. Gillis.

Cardiorespiratory effects produced by microinjecting L-glutamic acid into medullary nuclei associated with the ventral surface of the feline medulla. *Brain Research*, 381:281-288 (1986).

13. Gordon, F.J. Aortic baroreceptor reflexes are mediated by NMDA receptors in caudal ventrolateral medulla. *Am. J. Physiol.*, 252:R628-R633 (1987).
14. Giuliano, R., D.A. Ruggiero, S. Morrison, P. Emsberger, and D.J. Reis. Cholinergic regulation of arterial pressure by the C1 area of the rostral ventrolateral medulla. *J. Neurosci.*, 9(3):923-942 (1989).
15. Guyenet, P.G., T.M. Filtz, and S.R. Donaldson. Role of excitatory amino acids in rat vagal and sympathetic baroreflexes. *Brain Research*, 407:272-284 (1987).
16. Honoré, T., S.N. Davies, J. Drejer, E.J. Fletcher, P. Jacobsen, D. Lodge, and F.E. Nielsen. Quinoxalinediones: Potent competitive non-NMDA glutamate receptor antagonists. *Science*, 241:701-703 (1988).
17. Jung, R., E.N. Bruce, and P.G. Katona. Tonic and baroreflex effects on arterial pressure and ventilation of pentobarbital and nicotine on the rat ventral medullary surface. *Brain Research*, 485:399-402 (1989).
18. Kubo, T. and M. Kihara. N-Methyl-D-aspartate receptors mediate tonic vasodepressor control in the caudal ventrolateral medulla of the rat. *Brain Research*, 451:366-370 (1988).
19. Lawing, W.L., D.E. Millhorn, D.A. Bayliss, J.B. Dean, and A. Trzebski. Excitatory and inhibitory effects on respiration of L-glutamate microinjected superficially into the ventral aspects of the medulla oblongata in cat. *Brain Research*, 435:322-326 (1987).
20. Leone, C., and F.J. Gordon. Blockade of non-N-methyl-D-aspartic acid (NMDA) receptors in the nucleus of the tractus solitarius (NTS) eliminates aortic baroreflexes (Abstract). *Soc. Neurosci. Abstr.*, 15:1178 (1989).
21. McCrimmon, D.R., J.L. Feldman, and D.F. Speck. Respiratory motoneuronal activity is altered by injections of picomoles of glutamate into cat brain stem. *J. Neurosci.*, 6(8):2384-2392 (1986).
22. Mitra, J., N.R. Prabhakar, J.L. Overholt, and N.S. Chemiack. Respiratory and vasomotor responses to focal cooling of the ventral medullary surface (VMS)

of the rat. *Resp. Physiol.*, 74:35-48 (1988).

23. Moss, I.R., M. Denavit-Saubié, F.L. Eldridge, R.A. Gillis, M. Herkenham, and S. Lahiri. Neuromodulators and transmitters in respiratory control. *Fed. Proc.*, 45:2133-2147 (1986).
24. Onimaru, H., A. Arata, and I. Homma. Primary respiratory rhythm generator in the medulla of brainstem-spinal cord preparation from newborn rat. *Brain Research*, 445:314-324 (1988).
25. Onimaru, H., A. Arata, and I. Homma. Electrophysiological properties and localization of inspiratory pattern generator in medulla isolated from newborn rats (Abstract). *Jpn. J. Physiol.*, 39:S249 (1989).
26. Paxinos, G. and C. Watson. The rat brain in Stereotaxic Coordinates, Academic Press Inc, Florida, (1986).
27. Saether, K., G. Hillaire, R. Monteau. Dorsal and ventral respiratory groups of neurons in the medulla of the rat. *Brain Research*, 419:87-96 (1987).
28. Saji, M. and M. Miura. Thoracic expiratory motor neurons of the rat: localization and sites of origin of their premotor neurons. *Brain Research*, 507: 247-253 (1990).
29. Somogyi, P., J.B. Minson, D. Morilak, I. Llewellyn-Smith, J.R.A. McIlhinney, and J. Chalmers. Evidence for an excitatory amino acid pathway in the brainstem and for its involvement in cardiovascular control. *Brain Research*, 496:401-407 (1989).
30. Urbanski, R.W. and H.N. Sapru. Evidence for a sympathoexcitatory pathway from the nucleus tractus solitarii to the ventrolateral medullary pressor area. *J. Auton. Nerv. Syst.*, 23:161-174 (1988).
31. Urbanski, R.W. and H.N. Sapru. Putative neurotransmitters involved in medullary cardiovascular regulation. *J. Auton. Nerv. Syst.*, 25:181-193 (1988).
32. Willette, R.N., S. Punnen, A.J. Krieger, and H.N. Sapru. Interdependence of rostral and caudal ventrolateral medullary areas in the control of blood pressure. *Brain Research*, 321:169-174 (1984).
33. Yamada, K.A., J. M. Dubinsky, and S.M. Rothman. Quantitative physiological

characterization of a quinoxalinedione non-NMDA receptor antagonist. J. Neurosc., 9(9):3230-3236 (1989).

Table 1 Baseline mean arterial pressure (MAP) and change in MAP on aortic depressor nerve stimulation (BPR) before and after bilateral microinjection of kynurenic acid (KYN), 2-amino-5-phosphonovaleric acid (2APV), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) in the caudal ventrolateral depressor area.

		KYN (5 nmol) n = 4	KYN (1.58 nmol) n = 4	2APV (2.7 nmol) n = 5	CNQX (0.257 nmol) n = 5
Baseline MAP (mmHg)	BEFORE	85.6 ± 7.5	90.0 ± 18.3	95.2 ± 5.1	119.1 ± 11.2
	AFTER	163.0 ± 18.6*	178.5 ± 12.7*	138.7 ± 14.1*	120.1 ± 13.1
BPR (mmHg)	BEFORE	- 29.5 ± 4.0	-31.6 ± 11.6	-37.4 ± 5.0	-38.5 ± 9.6
	AFTER	+20.4 ± 11.7*	+12.0 ± 2.1*	+7.0 ± 2.3*	+5.3 ± 5.0*

* indicates significantly different after than before drug injection at $P \leq 0.05$.
Values are mean ± s.e.m.

Fig. 1 Typical systemic arterial pressure (SAP), respiratory flow (FLOW) and tidal volume (VT) responses to unilateral KYN (5 nmol in 50 nl) microinjection in the VLDA.

Fig. 2 Time profiles for 4 rats of mean arterial pressure (MAP), ventilation ($\dot{V}_E$), respiratory frequency (F), and tidal volume (VT) after unilateral microinjection of 5 nmol KYN in the CAUDAL sites. CNT = before microinjection. 6 breath averages every ten seconds. Each profile represents microinjections at one site per rat.

Fig. 3 Baro-pressure response to supramaximal aortic depressor nerve stimulation before (left) and after (right) bilateral microinjection of KYN (5 nmol) in the VLDA. Arrows indicate stimulus on and off.

Fig. 4 Typical SAP, FLOW, and VT responses to unilateral KYN (1.58 nmol in 31 nl) microinjection in the VLDA. (See Fig. 1 for abbr.)

Fig. 5 Time profiles for 4 rats of MAP, $\dot{V}E$, F, and VT after unilateral microinjection of 1.58 nmol KYN in the ROSTRAL (left panel) and CAUDAL (right panel) sites. (See Fig.2 for abbr.)

Fig. 6 Typical SAP, FLOW, and VT responses to unilateral 2APV (2.7 nmol) microinjection in the VLDA.

Fig. 7 Time profiles for 5 rats of MAP, VE, F, and VT after unilateral microinjection of 2.7 nmol 2APV in the ROSTRAL (left panel) and CAUDAL (right panel) sites. (See Fig. 2 for abbr.)

Fig. 8 Typical SAP, FLOW, and VT responses to unilateral CNQX (0.257 nmol) microinjection in the VLDA. (See Fig. 1 for abbr.)

Fig. 9 Time profiles for 5 rats of MAP, $\dot{V}E$, F, and VT after unilateral microinjection of CNQX in the ROSTRAL (left panel) and CAUDAL (right panel) sites. (See Fig. 2 for abbr.)

Fig. 10 Coronal sections indicating ROSTRAL (left panel), and CAUDAL (right panel) ventrolateral medullary sites where KYN, 2APV, and CNQX were microinjected. Each circle represents one site. Refer text for rostro-caudal distances. Abbr. 4V fourth ventricle, 7 facial nu., 12 hypoglossal nu., DC dorsal cochlear nu., IO inferior olive, LRt lateral reticular nu., Sol solitary tract nu., Sp5 spinal trigeminal nu..

CHAPTER VII

CONCLUSIONS AND DISCUSSION

OVERVIEW OF RESULTS

This research was designed to investigate the ventral medullary neural organization for mediating the baroreflex inhibition of blood pressure and ventilation. The specific hypotheses tested were:

- (1) Neural structures near the ventral medullary surface (VMS) and deeper in the rostral and caudal ventrolateral medulla (RVLM and CVLM respectively) that affect steady state vasomotor tone and ventilation are also part of the baro-pressure reflex and the baro-ventilatory reflex pathways, and
- (2) The baroreflexes are mediated by GABA_A receptors and *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors.

Table 1 qualitatively summarizes all the results of this research. The results of Chapter IV and V partly confirmed both hypotheses 1 and 2. Neural structures near the ventral medullary surface (VMS) of the rat were shown to influence both arterial pressure and ventilation. It was also confirmed that the control of basal arterial pressure and mediation of the baro-pressure reflex are, at least in part, through GABA_A receptors. However, we found that contrary to our hypothesis, neural structures near the VMS do not affect the baroreflex inhibition of ventilation.

Additionally, the tonic control of ventilation in the rat is not significantly mediated via GABA_A receptors on these neural structures.

Results from the experiments of Chapter III affirmed that deeper neural structures in the rostral ventral medulla (RVLM) of the dog can affect both arterial pressure and ventilation. Hypothesis 1 was supported by the results of these experiments since the RVLM neural structures of the dog were found to play a role in both the baro-pressure and baro-ventilatory reflexes.

Experimental results from Chapter VI confirm that structures in the caudal ventrolateral medulla (CVLM) affect control of arterial pressure and form an obligatory synapse for the baro-pressure reflex. Our 2nd hypothesis was corroborated as both NMDA receptors and non-NMDA receptors were found to be involved in mediating the baro-pressure reflex. It was also found that neural structures in the CVLM are essential for sustaining ventilation. Unilateral microinjections of kynurenic acid, a non-specific glutamatergic antagonist, caused respiratory arrest in spontaneously breathing rats (increase in frequency, but major drop in tidal volume), and raised the tonic blood pressure. Bilateral block abolished the baro-pressure reflex. Because of the respiratory arrest the baro-ventilatory reflex could not be studied. Further experiments to delineate the receptor types mediating these responses indicated that ventilation is sustained by both NMDA and non-NMDA receptor activation, while the increase in tonic blood pressure is only mediated via NMDA receptors.

The results make new contributions to our understanding of: (a) ventral medullary pathways that mediate the baroreflex inhibition of ventilation, (b) the role of caudal ventral medullary structures in sustaining ventilation, and (c) the putative receptor types involved in both the steady-state control of blood pressure and ventilation, and the baroreflex inhibition of blood pressure.

DISCUSSION

The conclusions are discussed briefly using schematics based on experimental studies performed in rats, cats, rabbits, and dogs, as discussed in the literature review in Chapter II, with the addition of the results obtained in the present research. All neural populations and pathways depicted in the schematics have not been studied in each of the above mentioned species. Similarly, different techniques and methodologies have been used within a species to determine the role of a neural population subserving cardio-respiratory control. However, the literature review suggests that at least some of the neural organization in the medulla for cardio-respiratory control is similar in different mammalian species hence, assuming an analogous organization of the major neural populations in the mammalian species, results obtained in our experiments can be summarized using these schematics.

Figure 1 shows a schematic of the neural structures underlying the VMS. Application of drugs to the VMS can affect both neural structures adjacent to the VMS and deeper structures depending on the diffusion of the applied drugs. The

neural structures that form the ventrolateral pressor area (VLPA) in the RVLM are closer to the VMS than are those that form the ventrolateral depressor area (VLDA) in the CVLM. Therefore, assuming the same depth of diffusion from the rostral and caudal surfaces, drug applications on the VMS that are located above the RVLM can affect a greater population of the VLPA cardiovascular neurons than those centered above the VLDA. Results from our experiments in Chapters IV and V suggest that neural structures of the VLPA were affected preferentially. Additionally, the observation that the baro-pressure reflex can be only partly blocked by VMS application of different drugs suggests that the drugs did not diffuse deep enough to act on a majority of the VLPA neurons since the VLPA neurons are considered to provide the final sympathetic outflow.

Immediately adjacent to the VMS lies a major concentration of putatively chemosensitive neurons that affect ventilation. However, the ventral respiratory group neurons are deeper and lie in a region spanning the VLDA neurons in the CVLM and the caudal border of the VLPA neurons in the RVLM. Thus VMS drug applications can affect tonic ventilation by actions on both the superficial and deeper neural populations, the degree of relative effectiveness depending upon the depth of drug diffusion. The finding that the baro-ventilatory reflex is not significantly affected by VMS drug applications suggests that, although chemosensitive neural structures near the VMS in the rat can effect baseline ventilation, these neural populations do not take part in the baroreflex inhibition of ventilation. Hence, the neural structures mediating

the baro-ventilatory reflex probably lie deeper in the ventral respiratory group, and therefore may not have been accessed by surface drug applications.

Deeper structures in the rostral and caudal ventrolateral medulla that affect vasomotor tone are also known to mediate the baro-pressure reflex. Since the ventral respiratory group neurons are juxtaposed with these neural structures (Fig. 1) we hypothesized that the neural structures that are known to mediate the baro-pressure reflex may also be part of the baro-ventilatory pathway.

Figure 2 illustrates the known pathways mediating the baro-pressure reflex, as well as speculative pathways that may subserve the baro-ventilatory reflex. Although the VLPA neural structures have been shown to be part of the baro-pressure pathway in the rat and rabbit, our results from focal cooling experiments in the dog confirmed that, like in other mammalian species, neural structures in the RVLM of the dog also play a role in the baro-pressure reflex. One speculative pathway for the baro-ventilatory reflex involves connections between the NTS and the dorsal respiratory group. Though the possible contribution of dorsal medullary pathways to the baro-ventilatory reflex cannot be ruled out, the results from the focal cooling experiments in the dog show that neural structures in the RVLM of the dog do play a role in the baro-ventilatory reflex (Chapter III). The baro-ventilatory reflex in the dog could thus be mediated by direct inhibitory projections from the nucleus tractus solitarius (NTS) to VRG neurons that are in the cooled RVLM area, or it could be mediated via connections from the VLPA cardiovascular neurons that are part of the baro-pressure

pathway to the VRG neurons. Because the rostral ventrolateral medullary VLPA neurons and part of the VRG neurons are juxtaposed, it seems plausible that, if functional connections exist from the VLPA neurons to the VRG neurons, focal cooling of the former would block not only the baro-pressure pathway but also the baro-ventilatory pathway. If in the dog, like in the rat, surface applications of drugs were found not to attenuate the baro-ventilatory reflex, the likelihood of direct projections from the NTS to neurons in the VRG would be strengthened.

Neural structures in the CVLM of the rat are known to be part of the baro-pressure pathway. To test our hypothesis that these structures are part of the baro-ventilatory pathway, and that excitatory amino acid (EAA) receptors mediate the reflex, the activity of the VLDA neurons of spontaneously breathing anesthetized rats was blocked by microinjecting kynurenic acid, a broad-band excitatory amino-acid antagonist. However, in addition to raising arterial pressure, the unilateral microinjection of kynurenic acid also caused ventilatory arrest. The ventilatory cessation was mainly due to a decrease in tidal volume suggesting that neurons affecting the development of respiratory burst pattern are either present in this region or are strongly affected by the neural substrates in this region. Since unilateral microinjections caused such profound affects, strong coupling between the two medullary halves is suggested. Further experiments indicated that activation of both NMDA and non-NMDA receptors was necessary to sustain ventilation. The ventilatory arrest, unfortunately, prevented further study of the role of CVLM neurons

in the baro-ventilatory reflex.

The baro-pressure reflex was abolished after bilateral microinjections of kynurenic acid into the CVLM. The role of RVLM and CVLM structures and putative excitatory amino acid receptors in control of basal blood pressure and the baro-pressure reflex are summarized in Figure 3. This schematic is a subset of Figure 2 and expands the box marked VLDA in Figure 2. It presents both the results obtained from microinjections in the CVLM of the rat, and data obtained from existing literature. Literature results show that both the RVLM and the CVLM neural structures in the rat are part of the baro-pressure reflex. Microinjection studies confirmed that the CVLM neurons form an obligatory synapse in the pathway mediating the inhibitory effect of the baro-pressure reflex and that these neural structures have NMDA receptors. We also found that neural structures of the CVLM having non-NMDA receptors are part of the baro-pressure reflex pathway, and that selective block of either the NMDA or non-NMDA receptors was sufficient to block the inhibitory effect of the baro-pressure reflex. Since the net baroreflex effect on arterial pressure results from the balance between the direct excitatory input from the NTS to the rostral pressor neurons and the indirect inhibitory input from the caudal depressor neurons, blocking one type of receptors is apparently sufficient to allow the direct excitatory input to dominate.

The effects of EAA receptor blockade on steady-state control of baseline arterial pressure and the transient baro-pressure reflex could be separated. On

blocking the EAA receptors in the VLDA with kynurenic acid, the baseline blood pressure increased and the baro-pressure reflex was abolished. However, the baseline blood pressure recovered before the baro-pressure reflex was reestablished. Additionally, selective NMDA receptor block increased the basal blood pressure. These results suggest two possibilities 1) that there exists a separate population of neurons with NMDA receptor sites that directly or indirectly provides a tonic inhibition of sympathetic neurons of the VLPA independent of baroreceptor mechanisms, or 2) that the same VLDA neuronal population that mediates the baro-pressure inhibition of the sympathetic neurons also receives a tonic input drive mediated by NMDA receptor sites. In this latter case, secondary mechanisms would have to be invoked to account for the recovery of tonic blood pressure before the recovery of the baro-pressure reflex.

Though microinjections of excitatory amino acids in the CVLM affected both arterial pressure and ventilation, the effects could sometimes be separated. For example, non-NMDA receptor block by CNQX microinjections did not affect baseline blood pressure but caused respiratory arrest. Also, the initial increase in baseline arterial pressure on kynurenic acid microinjections returned to control much faster than recovery of ventilation. These results suggest that neural populations affecting basal control of arterial pressure may be different from those affecting ventilation even though they are co-mingled.

In summary: (a) Vasomotor tone and ventilatory drive are affected by neural

structures of the ventrolateral medulla near the ventral surface in rats. These structures participate in the baro-pressure reflex, but contrary to our hypothesis are not involved in the baro-ventilatory reflex; (b) Although GABA_A receptors on neural structures near the VMS of the rat mediate control of the arterial pressure baseline and may be also involved in the baroreflex control of blood pressure they are not important in ventilatory control; (c) Neural structures in the rostral ventrolateral medulla of the dog influence baseline arterial pressure and ventilation and they also play a role in the baro-pressure and baro-ventilatory reflexes; (d) Even unilateral block of neural structures in the CVLM of the rat resulted in ventilatory cessation, therefore they are essential for sustaining ventilation; (e) CVLM neural structures of the rat provide an inhibitory influence to the sympathetic neurons that control blood pressure and are an obligatory synapse of the baro-pressure pathway; (f) Both NMDA and non-NMDA receptor activation in the CVLM is necessary to sustain ventilation; and (g) Only NMDA receptors mediate control of baseline blood pressure while both NMDA and non-NMDA receptors mediate the baro-pressure reflex.

Table 1 Qualitative summary of the principal results from different experiments. Responses calculated on a percentage basis were used for making the summary. '-' indicates no data available, 'o' indicates no affect, down arrow indicates decrease, up arrow indicates increase. Number of arrowheads indicate strength of response. 2APV - specific NMDA antagonist, BIC - Bicuculline (GABA_A antagonist), CNQX (specific non-NMDA antagonist), BPR - Baro-pressure reflex, BVR - Baro-ventilatory reflex, CVLM - caudal ventrolateral medulla, KYN - Kynurenic acid (non-specific glutamatergic antagonist), MAP - Baseline mean arterial pressure, RVLM - rostral ventrolateral medulla, $\dot{V}_E$ - baseline ventilation,

Neural Struct. (Species)	Techniq.	DRUG	MAP	$\dot{V}_E$	BPR	BVR
VMS (RAT)	Surface Appl.	PB	↓↓↓	↓	↓	o
		NIC	↓	↑↑	o	o
		BIC	↑↑	o	↓	o
RVLM (DOG)	Focal Cooling (Unilat.)	-	↓	o	↓	↓
RVLM (RAT)	Microinj. (Unilat.)	KYN	o	o	-	-
		2APV	o	o	-	-
		CNQX	o	o	-	-
CVLM (RAT)	Microinj. (Unilat.)	KYN	↑	↓↓↓	↓↓↓	-
		2APV	↑	↓↓↓	↓↓↓	-
		CNQX	o	↓↓↓	↓↓↓	-

Fig 1. Schematic representation of neural structures under the ventral medullary surface (VMS). The surface demarcations, M, S, and L are based on studies in cats. Other neural pools and demarcations are mainly based on studies in rats. The VRG though partly overlapping extends lateral to the VLPA and VLDA neurons. CHEMOSENSITIVE NEURONS - Putative maximum concentration of chemosensitive neurons, CVLM - Caudal Ventrolateral Medulla, RVLM - Rostral Ventrolateral Medulla, VLDA - Ventrolateral depressor area, VLPA - Ventrolateral pressor area, L - Loeschke's area, M - Mitchell's area, S - Schlaefke's area.

Fig 2. Schematic representation for baro-reflex pathways. Solid lines indicate known baro-pressure reflex pathways. Dashed lines with ? indicate speculative baro-ventilatory pathways not examined in this thesis. Dashed lines indicate baro-ventilatory pathway suggested by our results. DRG - Dorsal Respiratory Group, IML - Intermediolateral column, NTS - Nucleus Tractus Solitarius, PMN - Phrenic Motoneurons, VLDA - Ventrolateral Depressor Area, VLPA - Ventrolateral Pressor Area, VRG - Ventrolateral Respiratory Group, + excitatory, - inhibitory.

Fig 3. Schematic representation for control of arterial pressure. Solid thin lines indicate baroreflex pathway, solid thick lines indicate tonic inputs, Dashed lines indicate pathways suggested by our results, and bold letters indicate putative receptors suggested by our results. GLU - Glutamatergic, GR - GABAergic. See Fig. 1 for other abbreviations.

CHAPTER VIII

FUTURE WORK

This concluding chapter presents suggestions for new research avenues which were motivated by experimental results presented previously. This chapter also presents a qualitative neural network model that was developed to explore the roles of speculative connections between ventral medullary neural pools for generating the respiratory pattern. The model qualitatively reproduced the results obtained in Chapter VI of unilateral microinjections of NMDA and non-NMDA antagonists in the rat caudal depressor area. It assisted in formulating the proposed future experiments.

Neural network model for respiratory pattern generation

The neural circuit

The neural network model is shown in Figure 1. The general structure of the model was motivated by [6] which suggests that pacemaker neurons provide the rhythm generation, while interconnections between several other identified neuron pools in the ventral respiratory group shape the respiratory pattern. We speculated that results from our experiments described in Chapter VI may be explained by such a model.

The network is bilaterally symmetrical and each neural pool is modeled by a

representative neuron. Each medullary half has a pacemaker (P), which provides the basic respiratory rhythm, and inspiration related neuronal groups that shape the respiratory pattern. For the purposes of this model expiration is considered to occur passively, and no neural pools for expiration are included. The inspiratory groups used in the model include an early inspiratory group (EI), a late inspiratory group (LI), an inspiratory bulbospinal group (I), and a phrenic motoneuron group (PMN). The interconnecting pathways were chosen based on the literature as reviewed in Chapter II. Results from Chapter VI indicated that unilateral microinjections of excitatory amino acid antagonists into the CVLM of the rat not only decreased tidal volume but also affected the respiratory rate. Hence, speculative ipsilateral feedback connections to the pacemaker from the propriobulbar EI and LI interneuron groups were added. These are shown by dashed lines. Since EI neurons may provide the initial burst for inspiratory ramp generation, they were modeled as receiving direct input from the ipsilateral pacemaker neuron.

The neuron model

The representative neuron for each pool is based on a model presented in [2]. Figure 2 shows a schematic representation. Each neuron is modeled as a capacitive membrane of fixed conductance while inputs to the neuron can be either tonic or synaptic currents. Tonic input currents are constant currents which are injected into a neuron throughout the duration of a simulation run. Any neuron can receive

synaptic input from any other neuron; the amount of current injected at each synapse is determined by the output level of the presynaptic neuron and is scaled by the synaptic weight. Thus, the set of neurons is described by a set of coupled first-order differential equations:

$$C_i \, dV_i/dt = \sum (w_{ij} * V_{outj}) + I_{tonic_i} - V_i/R_i \quad i,j = 1,2,...,n$$

where:

V_i =	membrane voltage level of neuron i
V_{outi} =	output voltage level of neuron i
C_i =	membrane capacitance of neuron i
R_i =	membrane resistance of neuron i
I_{tonic_i} =	tonic current injected into neuron i
w_{ij} =	weighting factor for synapse from neuron j onto neuron i

The output voltage of each neuron is determined by passing the voltage level of the neuron through a nonlinear sigmoidal function that results in a value between 0 and 1:

$$V_{outi} = f(V_i) = 1 / (1 + \exp (-2 * g_i * (V_i - V_{thri})))$$

where g_i is a gain which determines the slope of the output function, and V_{thri} is the value of V_i at which $V_{outi} = 0.5$.

The pacemaker neuron has all the properties described above and two additional intrinsic currents which cause the membrane to oscillate [1]. One is a

continuously depolarizing current and the other is a phasic hyperpolarizing current. The hyperpolarizing current turns on when the membrane voltage goes above a certain threshold level ('on threshold') and turns off when the membrane voltage has decreased below another threshold voltage ('off threshold'). The equations describing the intrinsic currents are:

$$I_{\text{int}} = I_{\text{dep}} + I_{\text{hyp}}$$

$$I_{\text{hyp}} = g_{\text{hyp}} * (E_{\text{hyp}} - V_i) \quad \text{if } V_i \geq \text{on threshold}$$

$$I_{\text{hyp}} = 0 \quad \text{if } V_i \leq \text{off threshold}$$

where:

I_{int} = intrinsic current

I_{dep} = depolarizing current

I_{hyp} = hyperpolarizing current

g_{hyp} = conductance of channel for hyperpolarizing current

E_{hyp} = reversal potential for the hyperpolarizing current

V_i = membrane voltage for neuron i

The values for all the above variables were chosen arbitrarily such that a) the respiratory rate was approximately 120 breaths/min, b) EI and LI neurons fired during the appropriate phase of the respiratory cycle, and c) the shapes of the activity of the different pools were qualitatively similar to those shown in [3]. The ratio of the synaptic weights from the contralateral, and ipsilateral I neurons to the PMN was

based on anatomic data reported in the literature [5,11]. The parameter values for each of the neurons are given in Table 1, the pacemaker properties in Table 2, and the synaptic weights in Table 3.

Results of the simulations

The network was simulated using a computer program described in [1]. The program uses a fourth-order Runge-Kutta algorithm for integrating the differential equations. A step size of 0.01 seconds was initially determined as sufficient and was subsequently used in all simulation runs. Each simulation run lasted 10 seconds and 6 seconds of steady state data are shown below.

Figure 3 shows the output firing patterns of each of the neural pools under normal conditions. The pacemaker neuron burst is followed by an increase in the activity of the early inspiratory (EI) neurons, at the peak of which there occurs an increase in the inspiratory (I) activity. EI activity is terminated in about mid inspiration, and late inspiratory (LI) activity occurs at end inspiration. The phrenic motoneuron activity follows the I activity.

The microinjection data from Chapter VI was qualitatively simulated by severing connections and/or the tonic drive to the right neural pools. The unilateral microinjections could have affected excitatory inputs to the I, EI, and LI neurons as well as the tonic drives to them. Hence, the effects of blocking synaptic input to these neural pools were investigated in a series of simulation studies. Computer

outputs of some of these simulations are presented in Figures 3 through 7.

As shown in Fig. 4, blocking the synaptic input to the inspiratory neurons from the left early inspiratory neurons caused an increase in the rate of firing of the pacemakers and they became out of phase, i.e. the left pacemaker fired while the right pacemaker was silent and vice versa. This phase shift also caused the early inspiratory neurons to fire out of phase. The I neurons and hence the phrenic motoneuron began to oscillate at almost double the rate of the pacemakers. The output was also shallower. Similar responses were obtained if the tonic input to the right I neurons was blocked.

If the excitatory coupling from the left inspiratory neurons to the right inspiratory neurons was blocked (Fig. 5), the pacemakers continued to burst as in the previous case. The left and right pacemakers and the EI neurons fired in opposite phase. However, unlike in the previous case, the I neurons and hence the PMN neurons did not oscillate at double the frequency (the rate was only slightly higher than in Fig. 3) and the PMN output was almost abolished. The left I neurons continued to fire though with a lower amplitude. When the input from the left EI neurons and the left I neurons to the right I neurons was blocked responses similar to those in Fig. 5 were obtained.

The above simulations thus indicate that when the excitatory input to one set of I neurons from the contralateral I neurons is blocked, the PMN output is almost abolished bilaterally because of reduced amplitude of the PMN output. If the

excitatory coupling between the two I neural pools is intact and some other excitatory input to one of the I neural pools is blocked, the output firing frequency of the PMN neurons increases bilaterally, and simultaneously the amplitude of the PMN activity decreases. The shallow amplitude of the PMN activity may indicate a low tidal volume tidal. Note that the pacemaker activity is not significantly altered in any of the above simulations.

Simulations to mimic the potential block of excitatory input to the right EI neurons or the right LI neurons, on microinjection of the excitatory amino acid antagonists in the experiments, were also performed. In the simulation, blocking the early inspiratory input from the left to the right EI neurons caused responses similar to those obtained by blocking the input from the left EI neuron pool to the right I neuron pool, i.e increased rate of oscillation of the I and PMN neurons, with a very shallow output. However, blocking the excitatory input to the right late inspiratory neurons from the right I neurons prevented termination of the inspiratory activity (Fig. 6) which was not similar to the pattern of respiratory cessation observed in our experiments.

If the left EI input to the right EI and I neurons, and the left I input to the right I neurons was blocked, the I neuron activity, the LI neuron activity, and the PMN activity were all bilaterally suppressed (Fig. 7). The pacemakers continued to burst.

Though other possibilities are not ruled out, the simulations shown in Figures

3 and 4 could explain our results of unilateral microinjection of the NMDA and non-NMDA antagonists into the CVLM obtained in Chapter VI. Microinjections of the non-NMDA blocker CNQX caused respiratory arrest which in three cases was preceded by an increase in the respiratory frequency. From the simulations this could be akin to the synaptic block of input from the left EI neurons to the right I neurons. The rapid shallow PMN output would appear as an increase in respiratory frequency, but the shallow output could result in a non-functional tidal volume and hence the animal would go into ventilatory arrest. However, microinjections of the NMDA blocker 2APV showed an almost negligible change in respiratory frequency and the tidal volume was reduced resulting in loss of ventilation. The simulation shown in Fig. 5 where synaptic input from the left I neurons to the right I neurons is blocked mimics this condition since there was a loss of PMN output without much change in the frequency of firing of I or PMN neurons. The combination of the above two cases could explain the loss of sustainable ventilation observed on kynurenic microinjection. Thus the transient increase in respiratory frequency observed experimentally on kynurenic acid microinjection may be due to an initial action of this broad band antagonist on non-NMDA receptors mediating the connection between the contralateral EI neurons and the I neurons. If this were then followed with an action on the NMDA receptors mediating the synaptic input from the contralateral I neurons, ventilation would be completely arrested. In the model, the neural groups were dependent on tonic input currents for their activation, however, block of these tonic

drives was not necessary to cause ventilatory cessation.

The simulation examples shown indicate that the cross connections between the two medullary halves are important for maintaining normal respiratory pattern generation. They also suggest possible connections that may cause respiratory behaviors such as apnea due to prolongation of inspiration (Fig. 6) which were not seen in our experiments.

In rabbits after splitting the medulla, each medullary half continues to produce the respiratory rhythm independently [8]. However, in the cat it is very difficult to get this independent rhythm and it is dependent on strong external excitatory stimuli to respiratory neurons [4]. Since our results suggest strong cross-connections between the two medullary halves, it would be of interest to determine the effect of medullary splitting. We therefore simulated this in the model. PMN output and I neuron activity was bilaterally abolished when the coupling between the contralateral I neuron pools and their input from the contralateral EI neurons was interrupted (Fig. 8). This suggests that, unlike in the rabbit, each medullary half of the rat may not be capable of sustaining inspiratory output. Note that the pacemaker activity was not interrupted.

Suggestions for future research

Baro-ventilatory pathways

The baro-ventilatory pathways remain to be determined unequivocally. As

discussed in Chapter VII, though neural structures in the RVLM of the dog appear to play a role in the baro-ventilatory reflex, the role of these neural structures in the rat remain to be determined. The pathways for the baroreflex inhibition of respiratory neurons in this region are also undetermined. One possible method to determine if the baroreflex inhibition of ventilation occurs via "cardiovascular neurons" of the RVLM would be to block the activity of the cardiovascular neurons independent of the activity of respiratory neurons. If pharmacological methods are to be employed it will be necessary to use an agent that affects only the cardiovascular neurons and not the respiratory neurons. Though drugs to block the activity of the RVLM cardiovascular neurons are available, for example GABA, muscarinic type 2 receptor blocker AFDX-116 (both of which block the excitation of RVLM neurons [12,14]), their concomitant action on ventilation is not known. If these drugs do not cause a substantial depression of ventilation then baro-ventilatory reflex could be determined before and after microinjections of these drugs into the RVLM in doses that block the baro-pressure reflex. Persistence of the baro-ventilatory reflex would imply that neural structures of the RVLM that are part of the baro-pressure pathway are not part of the baro-ventilatory pathway. Partial depression of the baro-ventilatory reflex would indicate that other regions also participate in mediating the baro-ventilatory reflex. Similar experiments could be done to determine if the caudal ventrolateral medullary (CVLM) neural structures are part of the baro-ventilatory pathway.

To determine if dorsal respiratory group (DRG) neurons are part of the baro-

ventilatory pathway, it would be necessary to affect them without affecting the cardiovascular neurons in the nucleus tractus solitarius (NTS), since this is the site for the first synapse of the baroreceptor afferents. If these neurons can be lesioned, chemically or electrically, such that the baro-pressure reflex remains intact then pre- and post-lesioning responses of the baro-ventilatory reflex can be compared to assess the role of the DRG in mediating this reflex. Extracellular recordings from the DRG neurons could also be done while the baroreceptors are activated to determine if these neurons are affected by the baroreceptor stimulus.

Respiratory rhythm versus pattern generation

Experiments described in Chapter VI where excitatory amino acid receptor antagonists were microinjected into the CVLM of the rat suggest several further studies. The formulation of these experiments was assisted by the results obtained in the simulations. In our experiments ventilation could not be sustained after unilateral microinjections of EAA receptor antagonists. Since ventilation obtained by measuring the tracheal air flow is a consequence of activity of the respiratory musculature for both inspiration and expiration as well as activity of the upper airways, the first step would be to determine the action of the receptor blockade on central inspiratory and expiratory activity. This can be done by recording the phrenic (for inspiratory activity) and recurrent laryngeal nerves (for inspiratory and expiratory activity) bilaterally. Bilateral recordings would also indicate the proportion of crossover

between the two medullary halves.

One could also determine if inspiratory neurons in the two medullary halves have excitatory reciprocal coupling by recording from inspiratory neurons from one half of the medulla before and after EAA blockade on the opposite CVLM. The simulations indicate that if there is a strong coupling between the two sides then the activity in the inspiratory neurons should be attenuated as shown in Fig. 4 and Fig. 6. The importance of cross-coupling and capability of each medullary half to maintain an independent rhythm should be assessed by the midline sectioning of the medulla.

The hypothesis regarding the action of the antagonists, as suggested by the simulations, could also be tested by sequentially microinjecting CNQX and 2APV unilaterally while recording from the contralateral I neurons, phrenic nerve activity, and the tidal volume. CNQX microinjections alone should cause increased rate of firing of the I neurons and the phrenic nerve activity, while tidal volume should be reduced. Once the animal is being ventilated, 2APV microinjections should block the higher firing rate of the I neurons and the phrenic activity.

It has also been suggested from experiments in the neonatal rat brainstem-spinal cord preparation that pacemaker cells in the RVLM are the source of the respiratory rhythm [9]. It has been shown that respiratory rhythm in facial nerves is sustained in spite of blocking phrenic nerve activity [7]. Hence, it would be of interest to determine if EAA blockade in the CVLM abolishes phrenic nerve activity

but not facial nerve activity. Again, bilateral recordings would provide information regarding crossover. As demonstrated in simulation, if the rhythm generator were separate from the pattern generator one could obtain ventilatory arrest without considerably changing the pacemaker activity. Results from all the experiments described above would further our understanding of the central organization for respiratory control.

Control of arterial pressure in pathological states

The experiments of Chapter VI also indicated a dichotomy in the tonic versus baroreflex control of arterial pressure by neural structures in the CVLM. This information is of potential importance in pathological states such as hypertension and heart-failure where a sympathoexcitatory state exists. It has been suggested that abnormalities in the baroreflex inhibition of sympathetic neurons may contribute to this state [10,13]. It is possible that the affects are not only due to attenuation of the baroreflex-mediated inhibition but also to attenuation of other inhibitory inputs that determine the basal activity of the sympathetic neurons. To determine if the baroreflex-mediated inhibition is attenuated in hypertension or heart-failure, experiments can be done in normal rats versus rats with hypertension or in heart failure (induced by myocardial infarction). The baroreflex effects on arterial pressure can be assessed without making changes in the tonic inhibition of the sympathetic neurons by microinjecting the non-NMDA blocker CNQX bilaterally in the caudal

depressor area. Since CNQX microinjections did not cause changes in the baseline blood pressure in normal rats, the resultant baroreflex would indicate if indeed the baroreflexes were attenuated without confounding effects due to changes in baseline.

REFERENCES

1. Abbas, J.J., A neural network pattern generator for movement in a simulated human leg. MSL-Cleveland VAMC Tech. Rpt. 11 (1990).
2. Beer, R.D., Chiel, H.J. and Sterling, L.S., Heterogeneous neural networks for adaptive behavior in dynamic environments. CWRU_CAISR Tech. Rpt. TR 89-101 (1989).
3. Botros, S.M. and E.N. Bruce. Neural network implementation of a three-phase model of respiratory rhythm generation. Biol. Cybern., 63:143-153 (1990).
4. Eldridge, F.L. and D. Paydarfar. Desynchronized respiratory rhythms and their interactions in cats with split brain stems. J. Physiol., 410:513-532 (1989).
5. Ellenberger, H.H. and J.L. Feldman. Monosynaptic transmission of respiratory drive to phrenic motoneurons from brainstem bulbospinal neurons in rats. J. Comp. Neurol., 269:47-57 (1988).
6. Feldman, J.L. and J.C. Smith, Cellular mechanisms underlying modulation of breathing pattern in mammals. Ann. of N.Y. Acad. Sci., 563:114-130, 1989.
7. Huang, Q. and W.M. St.John. Respiratory neural activities after caudal-to-rostral ablation of medullary regions. J.Appl. Physiol., 64(4):1405-1411, 1988.
8. Janczewski, W.A. and W.A. Karczewski. Respiratory effects of pontine, medullary and spinal cord midline sections in the rabbit. Respir. Physiol., 57:293-305 (1984).
9. Onimaru, H. and I. Homma, Respiratory rhythm generator neurons in medulla of brainstem-spinal cord preparation from newborn rat. Brain Research, 403: 380-384 (1987).
10. Packer, M. Neurohumoral interactions and adaptations in congestive heart failure. Circ., 77:721-730 (1988).
11. Saji, M. and M. Miura. Thoracic expiratory motor neurons of the rat: localization and sites of origin of their premotor neurons. Brain Research, 507:247-253 (1990).
12. Sapru, H.N. Cholinergic mechanisms subserving cardiovascular function in the

medulla and spinal cord. *Prog. Brain Research*, 81:171-179 (1989).

13. Smith, J.K. and K.W. Barron. GABAergic responses in ventrolateral medulla in spontaneously hypertensive rats. *Am. J. Physiol.*, 258(27):R450-R456 (1990).
14. Willette, R.N., A.J. Kriegger, P.P. Barcas, and H.N. Sapru. Medullary γ -Aminobutyric Acid (GABA) receptors and the regulation of blood pressure in the rat. *J. Pharmacol. Exp. Therap.*, 226(3):893-899 (1983).

Table 1. Parameter values for each of the neurons. Abbr. as in Fig. 1. l - indicates left neuron, r - indicates right neuron. The parameter values are the same for the left and right pair.

NEURON	R,	C,	INIT_V,	TONIC,	THR,	GAIN,
Pl,	0.5000,	0.5000,	0.0000,	2.2000,	6.9000,	10.0000,
Pr,	0.5000,	0.5000,	0.0000,	2.2000,	6.9000,	10.0000,
EIl,	0.1000,	1.0000,	1.0000,	7.0000,	2.5000,	2.5000,
EIr,	0.1000,	1.0000,	1.0000,	7.0000,	2.5000,	2.5000,
LIl,	0.2000,	0.3000,	0.0000,	4.0000,	4.5000,	1.5000,
LIr,	0.2000,	0.3000,	0.0000,	4.0000,	4.5000,	1.5000,
Il,	0.3000,	0.8000,	0.0000,	2.0000,	3.5000,	0.6000,
Ir,	0.3000,	0.8000,	0.0000,	2.0000,	3.5000,	0.6000,
PMNl,	0.1000,	0.5000,	0.0000,	3.0000,	2.6000,	0.8000,
PMNr,	0.1000,	0.5000,	0.0000,	3.0000,	2.6000,	0.8000,

Table 2. Pacemaker properties for the left and right pacemakers.

	Idep,	ghyp,	Ehyp,	Vonthr,	Voffthr
Pl,	23.0000,	0.9300,	-20.0000,	8.3000,	3.7500
Pr,	23.0000,	0.9300,	-20.0000,	8.3000,	3.7500

Table 3. Synaptic weights for different connections.

NEURON	(INPUT NEURON, WEIGHT)		
P1	E11,12.00,	L11,-5.00,	
Pr	E1r,12.00,	L1r,-5.00,	
E11	P1,26.00,	E1r,12.00,	
E1r	Pr,26.00,	E11,12.00,	
L11	E11,-5.00,	I1,30.00,	
L1r	E1r,-5.00,	I1,30.00,	
I1	L11,-110.00,	E1r,30.00,	Ir,40.00.
Ir	L1r,-110.00,	E11,30.00,	I1,40.00.
PMN1	I1,22.00,	Ir,28.00,	
PMNr	Ir,22.00,	I1,28.00,

Fig. 1 Neural network circuit for the respiratory pattern generating model. P - Pacemaker neuron, EI - Early Inspiratory neuron, LI - Late Inspiratory neuron, I - Inspiratory bulbospinal neuron, PMN - Phrenic Motoneuron; Subscript L indicates left medullary half, while subscript R indicates right medullary half. Solid line indicate pathways suggested by literature. Dashed lines indicate speculative pathways. Filled circles are inhibitions, Dashes are excitations.

Fig. 2 Schematic electrical equivalent representation of a neuron (See text for explanation). Inset shows output voltage of a neuron as a function of its membrane voltage.

Fig. 3 Normal neural activity of all the respiratory pools shown in Fig. 1. Abbr. as in Fig. 1.

Fig. 4 Effect of blocking synaptic input from left EI neurons to right I neurons.
Abbr. as in Fig. 1.

Fig. 5 Effect of blocking synaptic input from left I neurons to right I neurons. Abbr. as in Fig. 1.

Fig. 6 Effect of blocking synaptic input from right I neurons to right LI neurons.
Abbr. as in Fig. 1.

Fig. 7 Effect of blocking synaptic input from left EI neurons to right EI and I neurons, and blocking synaptic input from left I neurons to right I neurons. Abbr. as in Fig. 1.

Fig. 8 Effect of blocking synaptic input from the left EI neurons to the right I neurons, from the right EI neurons to the left I neurons, and the reciprocal inputs between the left and right I neurons. Abbr. as in Fig. 1.

BIBLIOGRAPHY

1. Abbas, James, J., A neural network pattern generator for movement in a simulated human leg. MSL-Cleveland VAMC Tech. Rpt. 11 (1990).
2. Adams, M., T. Chonan, N.S. Cherniack, and C. von Euler. Effects on respiratory pattern of focal cooling in the medulla of the dog. *J. Appl. Physiol.*, 65(5):2004-2010 (1988).
3. Agarwal, S.K, A.J. Gelsema, and F.R. Calaresu. Inhibition of rostral VLM by baroreceptor activation is relayed through caudal VLM. *Am. J. Physiol.*, 27:R1271-R1278 (1990).
4. Agarwal, S.K., A.J. Gelsema, and F.R. Calaresu. Neurons in rostral VLM are inhibited by chemical stimulation of caudal VLM in rats. *Am. J. Physiol.*, 257: R265-R270 (1989).
5. Arata, A., H. Onimaru, and I. Homma. Respiration-related neurons in the ventral medulla of newborn rats in vitro. *Brain Res. Bull.*, 24:599-604 (1990).
6. Barman, S.M. and G.L. Gebber. Lateral tegmental field neurons of cat medulla: A source of basal activity of ventrolateral medullospinal sympathoexcitatory neurons. *J. Physiol.*, 57(5):1410-1424 (1987).
7. Bonham, A.C. and I. Jeske. Cardiorespiratory effects of DL-homocysteic acid in caudal ventrolateral medulla. *Am. J. Physiol.* 256:H688-H696 (1989).
8. Beer, R.D., Chiel, H.J. and Sterling, L.S., Heterogeneous neural networks for adaptive behavior in dynamic environments. CWRU_CAISR Tech. Rpt. TR 89-101 (1989).
9. Blessing, W.W. and J.O. Willoughby. Depressor neurons in rabbit caudal medulla do not transmit the baroreceptor-vasomotor reflex. *Am. J. Physiol.*, 253(22):H777-H786 (1987).
10. Botros, S.M. and E.N. Bruce. Neural network implementation of a three-phase model of respiratory rhythm generation. *Biol. Cybern.*, 63:143-153 (1990).
11. Brody, M.J., R.H. Alper, T.P. O'Neill, and J.P. Porter. Central neural control of the cardiovascular system. In: A. Zanchetti and R.C. Tarazi (eds.), *Handbook of Hypertension*, Elsevier Science Pub. B.V., Amsterdam, Vol. 8,

p.1. (1986).

12. Brown, L.D. and P.G. Guyenet. Electrophysiological study of cardiovascular neurons in the rostral ventrolateral medulla in rats. *Circ. Res.*, 56:359-369, (1985).
13. Bruce, E.N. and N.S. Cherniack. Central chemoreceptors. *J. Appl. Physiol.*, 62:389-402 (1987).
14. Brunner, M.J., M.S. Sussman, A.S. Green, and A.K. Shoukas. Carotid sinus baroreceptor reflex control of respiration. *Circ. Res.*, 51:624-636, (1982).
15. Budzinska, K., C. von Euler, F.F. Kao, T. Pantaleo, and Y. Yamamoto. Effects of graded focal cold block in rostral areas of the medulla. *Acta. Physiol. Scand.*, 124:329-340 (1985).
16. Caverson, M.M., J. Ciriello, and F.R. Calaresu. Chemoreceptor and baroreceptor inputs to ventrolateral medullary neurons. *Am. J. Physiol.*, 247(16):R872-R879 (1984).
17. Chonan, T., E.M. Adams, C.v. Euler, and N.S. Cherniack. Effects of focal cooling in the ventrolateral medulla on chemoresponsiveness in dogs. *Resp. Physiol.*, 80:45-54 (1990).
18. Chungcharoen, D., M. DeBurgh Daly, E. Neil, and A. Schweitzer. The effect of carotid occlusion upon the intrasinus pressure with special reference to vascular communications between the carotid and vertebral circulations in the dog, cat, and rabbit. *J. Physiol.*, 117:56-76 (1952).
19. Ciriello, J., M.M. Caverson, and C. Polosa. Function of the ventrolateral medulla in the control of the circulation. *Brain. Res. Rev.*, 11:359-391 (1986).
20. Collinridge, G.L. and R.A.J. Lester. Excitatory amino acid receptors in the vertebrate central nervous system. *Pharmacol. Rev.*, 40(2):143-210 (1989).
21. Dailey, J.W. Sedative-hypnotic and anxiolytic agents. In: *Modern Pharmacology*. Charles R. Craig and Robert E. Stitzel (eds.) Little, Brown and Co., Boston. Chp. 32, p. 440-452 (1990).
22. Dampney, R.A.L. Functional organization of central cardiovascular pathways. *Clin. Exp. Pharmac. Physiol.*, 8:241-259 (1981).

23. Dev, N.B. and H.H. Loeschcke. Topography of the respiratory and circulatory responses to acetylcholine and nicotine on the ventral surface of the medulla oblongata. *Pflüg. Arch.*, 379:19-27 (1979).
24. Donohgue, S., R.B. Felder, D. Jordan and K.M. Spyer. The central projections of carotid baroreceptors and chemoreceptors in the cat: A neurophysiological study. *J. Physiol.*, 347:397-401 (1984).
25. Dove, E.L., P.G. Katona, Respiratory effects of brief baroreceptor stimuli in the anesthetized dog. *J. Appl. Physiol.*, 59:1258-1265 (1983).
26. Eldridge, F.L. and D.E. Millhorn. Central regulation of respiration by endogenous neurotransmitters and neuromodulators. *Ann. Rev. Physiol.*, 43:121-135 (1981).
27. Eldridge, F.L. and D. Paydarfar. Desynchronized respiratory rhythms and their interactions in cats with split brain stems. *J. Physiol.*, 410:513-532 (1989).
28. Ellenberger, H.H. and J.L. Feldman. Monosynaptic transmission of respiratory drive to phrenic motoneurons from brainstem bulbospinal neurons in rats. *J. Comp. Neurol.*, 269:47-57 (1988).
29. Ellenberger, H.H. and J.L. Feldman. Brainstem connections of the rostral ventral respiratory group of the rat. *Brain Research*, 513:35-42 (1990).
30. Ellenberger, H.H., J.L. Feldman, and W.Z. Zhan. Subnuclear organization of the lateral tegmental field of the rat. II: Catecholamine neurons and ventral respiratory group. *J. Comp. Neurol.*, 294:212-222 (1990).
31. Euler, C. VON. Brain-stem mechanisms for generation and control of breathing pattern. In: *Handbook of Physiology. The Respiratory system. Control of Breathing*. Bethesda, MD: Am. J. Physiol. Soc., sect. 3, vol II, chapt. 9, p.463-523 (1986).
32. Feldberg, W. and P.G. Guertzenstein, A vasodepressor effect of pentobarbitone sodium. *J. Physiol., Lond.* 224:83-103 (1972).
33. Feldman, J.L. and J.C. Smith, Cellular mechanisms underlying modulation of breathing pattern in mammals. *Ann. of N.Y. Acad. Sci.*, 563:114-130 (1989).
34. Feldman, J.L., J.C. Smith, D.R. McCrimmon, H.H. Ellenberger, D.F. Speck.

- Generation of respiratory pattern in mammals. In A.M. Cohen, S. Grillner, and S. Rossignol (eds.): *Neural Control of Rhythmic Movements in Vertebrates*. New York: Wiley and Sons, chapt. 3, p.73-100 (1988).
35. Gardiner, S.M. and T. Bennett. Endogenous vasopressin and baroreflex mechanisms. *Brain Research*, 11:317-334 (1986).
 36. Gatti, P.J., W.P. Norman, A.M. Taveira Dasilva, and R.A. Gillis. Cardiorespiratory effects produced by microinjecting L-Glutamic acid into medullary nuclei associated with the ventral surface of the feline medulla. *Brain. Res.*, 381:281-288 (1986).
 37. Giuliano, R., D.A. Ruggiero, S. Morrison, P. Ernsberger, and D.J. Reis. Cholinergic regulation of arterial pressure by the C1 area of the rostral ventrolateral medulla. *J. Neurosci.*, 9(3):923-942 (1989).
 38. Gordon, F.J. Aortic baroreceptor reflexes are mediated by NMDA receptors in caudal ventrolateral medulla. *Am. J. Physiol.*, 252(21):R628-R633 (1987).
 39. Granata, A.R., D.A. Ruggiero, D.H. Park, T.H. Joh, and D.J. Reis, Brain stem area with C1 epinephrine neurons mediates baroreflex vasodepressor responses. *Am. J. Physiol.*, 248(17):H547-H567 (1985).
 40. Gurtzenstein, P.G. and O.U. Lopes. Cardiovascular responses evoked from the nicotine sensitive area on the ventral surface of the medulla oblongata in the cat. *J. Physiol.*, 347:345-360 (1984).
 41. Guyenet, P.G., T.M. Filtz, and S.R. Donaldson. Role of excitatory amino acids in rat vagal and sympathetic baroreflexes. *Brain Research*, 407:272-284 (1987).
 42. Harvey, S.C., Hypnotics and sedatives. In A. Goodman Gilman, L.S. Goodman, T.W. Rall, F. Murad (Eds.), *The Pharmacological Basis of Therapeutics*, McMillan, New York, pp. 339-371 (1985).
 43. Hedner, J., T. Hedner, J. Jonason, and D. Lundberg. GABA-ergic mechanisms in central respiratory control in the anesthetized rat. *Naun. Schm. Arch. Pharmac.*, 317:315-320 (1981).
 44. Honoré, T., S.N. Davies, J. Drejer, E.J. Fletcher, P. Jacobsen, D. Lodge, and F.E. Nielsen. Quinoxalinedones: Potent competitive non-NMDA glutamate receptor antagonists. *Science*, 241:701-703 (1988).

45. Housley, G.D. and J.D. Sinclair. Localization by kainic acid lesions of neurons transmitting the carotid chemoreceptor stimulus for respiration in the rat. *J. Physiol.*, 406:99-114 (1988).
46. Huang, Z.S., G.L. Gebber, S.M. Barman, and K.J. Varner. Forebrain contribution to sympathetic nerve discharge in anesthetized cats. *Am. J. Physiol.*, 252(21):R645-R652 (1987).
47. Huang, Q. and W.M. StJohn. Respiratory neural activities after caudal-to-rostral ablation of medullary regions. *J. Appl. Physiol.*, 64(4):1405-1411 (1988).
48. Janczewski, W.A. and W.A. Karczewski. Respiratory effects of pontine, medullary and spinal cord midline sections in the rabbit. *Respir. Physiol.*, 57:293-305 (1984).
49. Jung, R., E.N. Bruce, and P.G. Katona. Tonic and baroreflex effects on arterial pressure and ventilation of pentobarbital and nicotine on the rat ventral medullary surface. *Brain Research*, 485:399-402 (1989).
50. Jung, R. and P.G. Katona. Cardiovascular and respiratory responses to slow ramp carotid sinus pressures in the dog. *J. Appl. Physiol.*, 68(4):1465-1474 (1990).
51. Keeler, J.R., C.W. Shults, T.N. Chase, and C.J. Helke. The ventral surface of the medulla in the rat: Pharmacological and autoradiographic localization of GABA-induced cardiovascular effects. *Brain Research*, 297:217-224 (1984).
52. Kleinbaum, D.G. and L.L. Kupper. *Applied regression analysis and other multivariable methods*. PWS, Publishers; Boston, Massachusetts, 1978.
53. Koepchen, H.P. Respiratory and cardiovascular "centers": Functional entirety or separate structures? In: *Central neurone environment and the control systems of breathing and circulation*. Springer-Verlag, Publisher; Heidelberg, 1983.
54. Kubo, T. and M. Kihara. N-methyl-D-aspartate receptors mediate tonic vasodepressor control in the caudal ventrolateral medulla of the rat. *Brain Research*, 451:366-370 (1988).
55. Kubo, T. and M. Kihara. Evidence for γ -aminobutyric acid receptor mediated modulation of aortic baroreceptor reflex in the nucleus tractus solitarii of the rat. *Neurosc. Lett.*, 89:156-160 (1988).
56. Lawing, W.L., D.E. Millhorn, D.A. Bayliss, J.B. Dean, and A. Trzebski. Excitatory and inhibitory effects on respiration of L-glutamate microinjected superficially into the ventral aspects of the medulla oblongata in cat. *Brain Research*, 435:322-326 (1987).

57. Leone, C. and F.J. Gordon. Blockade of non-*N*-methyl-D-aspartic acid (NMDA) receptors in the nucleus of the tractus solitarius (NTS) eliminates aortic baroreflexes (Abstract). Soc. Neurosci. Abstr., 15:1178 (1989).
58. Lim, R.K.S., C.N.Liu and R.L. Moffitt. A stereotaxic atlas of the dog's brain. Charles C. Thomas, Publisher, Springfield, Ill., USA, 1960.
59. Lipski, J., R.M. McAllen, and K.M. Spyer. The sinus nerve and baroreceptor input to the medulla of the cat. J. physiol., 251:61-78 (1975).
60. Long, S.E. and J. Duffin. The medullary respiratory neurons: a review. Can. J. Physiol. Pharmacol., 62(2):161-182 (1984).
61. Luft, F.C., Th. Unger, R.E. Lang, and D. Ganten. Chemical messengers and transmitters in the brain and cardiovascular regulation. In: A. Zanchetti and R.C. Tarazi (eds.), *Handbook of Hypertension*, Elsevier Science Pub. B.V., Amsterdam, Vol. 8, p.26. (1986).
62. Maass-Moreno, R. and P.G. Katona. Species dependence of baroreceptor effects on ventilation in the cat and the dog. J. Appl. Physiol., 67:2116-2124 (1989).
63. Malcolm, J.L., I.H. Sarelius, and J.D. Sinclair. The respiratory role of the ventral surface of the medulla studied in the anesthetized rat, J. Physiol., 307:503-515 (1980).
64. Marson, L. and A.D. Loewy. Topographic organization of substance P and monoamine cells in the ventral medulla of the cat. J. Auton. Nerv. System, 14:271-285 (1985).
65. Martin, R.L. and J.D. Sinclair. Kainic acid on the rat ventral medullary surface depresses hypoxic and hypercapnic ventilatory responses, Resp. Physiol., 80:55-70, (1990).
66. McAllen, R.M. Location of neurones with cardiovascular and respiratory function, at the ventral surface of the cat's medulla. Neuroscience, 18(1):43-49 (1986).
67. McAllen, R.M., J.J.Neil, and A.D.Loewy. Effects of kainic acid applied to the ventral surface of the medulla oblongata on vasomotor tone, the baroreceptor reflex and hypothalamic autonomic responses. Brain. Res., 238:65-76 (1982).
68. McCrimmon, D.R., J.L. Feldman, and D.F. Speck. Respiratory motoneuronal activity is altered by injections of picomoles of glutamate into cat brain stem. J. Neurosci., 6(8):2384-2392 (1986).

69. McWilliam, P.N. and S.L. Shepherd. A GABA-mediated inhibition of neurones in the nucleus tractus solitarius of the cat that respond to electrical stimulation of the carotid sinus nerve. *Neurosc. Lett.*, 94:321-326 (1988).
70. Millhorn, D.E. and F.L. Eldridge, Role of the ventrolateral medulla in regulation of respiratory and cardiovascular systems. *J. Appl. Physiol.*, 61(4):1249-1263 (1986).
71. Mitchell, R.A., H.H. Loeschcke, W.H. Massion, and J.W. Severinghaus. Respiratory responses mediated through superficial chemosensitive areas on the medulla. *J. Appl. Physiol.*, 18(3):523-533 (1963).
72. Mitra, J., N.R. Prabhakar, J.L. Overholt, and N.S. Cherniack. Respiratory and vasomotor responses to focal cooling of the ventral medullary surface (VMS) of the rat. *Resp. Physiol.*, 74:35-48 (1988).
73. Miura, M., K. Takayama, and J. Okada. Study of possible transmitters in the solitary tract nucleus of the cat involved in the carotid sinus baro-and chemoreceptor reflex. *J. Auton. Nerv. Sys.*, 19:179-188 (1987).
74. Moss, I.R., M. Denavit-Saubié, F.L. Eldridge, R.A. Gillis, M. Herkham, and S. Lahiri. Neuromodulators and transmitters in respiratory control. *Fed. Proc.* 45:2133-2147 (1986).
75. Onai, T., M. Saji, and M. Miura. Functional subdivisions of the nucleus tractus solitarii of the rat as determined by circulatory and respiratory responses to electrical stimulation of the nucleus. *J. Auton. Nerv. Sys.*, 21:195-202 (1987).
76. Onai, T., M. Saji, and M. Miura. Projections of supraspinal structures to the phrenic motor nucleus in rats studied by a horseradish peroxidase microinjection method. *J. Auton. Nerv. Sys.*, 21:233-239 (1987).
77. Onimaru, H., A. Arata, and I. Homma. Primary respiratory rhythm generator in the medulla of brainstem-spinal cord preparation from newborn rat. *Brain Research*, 445:314-324 (1988).
78. Onimaru, H., A. Arata, and I. Homma. Electrophysiological properties and localization of inspiratory pattern generator in medulla isolated from newborn rats. *Jpn. J. Physiol.*, 39:S249 (1989).
79. Onimaru, H. and I. Homma, Respiratory rhythm generator neurons in medulla of brainstem-spinal cord preparation from newborn rat. *Brain Research*, 403: 380-384 (1987).

80. Packer, M. Neurohumoral interactions and adaptations in congestive heart failure. *Circ.*, 77:721-730 (1988).
81. Paxinos, G. and C. Watson. The rat brain in Stereotaxic Coordinates, Academic Press Inc, Florida, (1986).
82. Richter, D.W. and H. Seller. Baroreceptor effects on medullary respiratory neurones of the cat. *Brain Research*, 86:168-171 (1975).
83. Richter, D.W. Generation and maintenance of the respiratory rhythm. *J. Exp. Biol.*, 100:93-107 (1982).
84. Ross, C.A., D.A. Ruggiero, D.H. Park, T.H. Joh, A.F. Sved, J. Fernandez-Pardal, J.M. Saavedra, and D.J. Reis. Tonic and voasomotor control by the rostral ventrolateral medulla: Effect of electrical or chemical stimulation of the area containing C1 adrenalin neurons on arterial pressure, heart rate and plasma catecholamines and vasopressin. *J. Neurosc.* 4(2):474-494 (1984).
85. Ross, C.A., D.A. Ruggiero, and D.J. Reis. Projections from the nucleus tractus solitarii to the rostral ventrolateral medulla. *J. Comp. Neurol.*, 242:511-534 (1985).
86. Saether, K., G. Hillaire, R. Monteau. Dorsal and ventral respiratory groups of neurons in the medulla of the rat. *Brain Research*, 419:87-96 (1987).
87. Saji, M. and M. Miura. Thoracic expiratory motor neurons of the rat: localization and sites of origin of their premotor neurons. *Brain Research*, 507:247-253 (1990).
88. Sapru, H.N. Cholinergic mechanisms subserving cardiovascular function in the medulla and spinal cord. *Prog. Brain Research*, 81:171-179 (1989).
89. Sapru, H.N., E. Gonzaelez, and A.J. Krieger. Aortic nerve stimulation in the rat: Cardiovascular and respiratory responses. *Brain Res. Bull.*, 6:393-398 (1981).
90. Sapru, H.N. and A.J. Krieger. Carotid and aortic chemoreceptor function in the rat. *J. Appl. Physiol.*, 42(3):344-348 (1977).
91. Schläefke, M. and H.H. Loeschcke. Lokalisation eines an der Regulation von Atmung und Kreislauf beteiligten Gebietes an der ventralen Oberfläche der Medulla oblongata durch Kalteblockade. *Pflugers Archiv.*, 297:201-220 (1967).
92. Schmid, K., G. Böhmer, and K. Gebauer. GABA_B receptor mediated effects on central respiratory system and their antagonism by phaclofen. *Neurosc. Lett.*, 99:305-310 (1989).

93. Smith, J.K. and K.W. Barron. GABAergic responses in ventrolateral medulla in spontaneously hypertensive rats. *Am. J. Physiol.*, 258(27):R450-R456 (1990).
94. Somogyi, P., J.B. Minson, D. Morilak, I. Llewellyn-Smith, J.R.A. McIlhinney, and J. Chalmers. Evidence for an excitatory amino acid pathway in the brainstem and for its involvement in cardiovascular control. *Brain Research*, 496:401-407 (1989).
95. Urbanski, R.W. and H.N. Sapru. Evidence for a sympathoexcitatory pathway from the nucleus tractus solitarii to the ventrolateral medullary pressor area. *J. Auton. Nerv. Syst.*, 23:161-174 (1988).
96. Urbanski, R.W. and H.N. Sapru. Putative neurotransmitters involved in medullary cardiovascular regulation. *J. Auton. Nerv. Syst.*, 25:181-193 (1988).
97. Willette, R.N., A.J. Kriegger, P.P. Barcas, and H.N. Sapru. Medullary γ -aminobutyric acid (GABA) receptors and the regulation of blood pressure in the rat. *J. Pharmacol. Exp. Therap.*, 226(3):893-899 (1983).
98. Willette, R.N., S. Punnen, A.J. Krieger, and H.N. Sapru. Interdependence of rostral and caudal ventrolateral medullary areas in the control of blood pressure. *Brain Research*, 321:169-174 (1984).
99. Yamada, K.A., W.P. Norman, P. Hamosh, and R.A. Gillis. Medullary ventral surface GABA receptors affect respiratory and cardiovascular function. *Brain Research*, 248:71-78 (1982).
100. Yamada, K.A., J.M. Dubinsky, and S.M. Rothman. Quantitative physiological characterization of a quinoxalinedione non-NMDA receptor antagonist. *J. Neurosci.*, 9(9):3230-3236 (1989).
101. Yamada, K.A., R.M. McAllen, and A.D. Loewy. GABA antagonists applied to the ventral surface of the medulla oblongata block the baroreceptor reflex. *Brain Research*, 297:175-180 (1984).