THE MEDULLARY REPRESENTATION OF THE BARORECEPTOR REFLEX

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ABSTRACT

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By

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The purpose of my research was an attempt to elucidate the neural pathways in the medulla involved in the baroreceptor reflex concentrating on the sympatho-inhibitory component and to ascertain the relative contribution of proposed medullary sites mediating this reflex. Past research in this area has yielded much conflicting data and interpretations as to the importance of various medullary sites involved in the reflex.

Stimulation techniques were employed in an attempt to characterize the cardiovascular responses elicited from proposed medullary sites in the baroreceptor reflex pathway. Stimulation of NTS produced a large depressor response as well as a pronounced bradycardia suggesting that both components of the baroreceptor reflex (sympatho-inhibition and vagal activation) were present at this site. Stimulation of RMN resulted in a large depressor response. The heart rate changes (small bradycardia) were shown to be mediated by sympatho-inhibitory fibers to the heart and thus, no vagal component of the reflex was present at this site. Similiar responses were obtained by electrical activation of the inferior olivary nucleus.

The second phase of the experiments involved ablation of various medullary sites in vagotonized cats in order to ascertain the importance

and contribution of the lesioned areas to the sympatho-inhibitory component of the baroreceptor reflex. Baroreceptor reflex activity was modified by the following techniques: BLCO, i.v. injection of NE, and stimulation of nuclear sites proposed to be in the pathway. Ablation of NTS prevented the compensatory reflex adjustments to baroreceptor activation. This was shown by the following: The pressor response to BLCO was significantly reduced; the depressor response to PMN stimulation was substantially potentiated; and mean blood pressure increased following ablation. The data obtained from PMN ablation experiments indicated that the lesioned area did not contribute significantly to the sympatho-inhibitory pathway of the baroreceptor reflex: The pressor response to BLCO was reduced only 10% following bilateral ablation of this nucleus. Midline ablation data revealed that the classic "depressor area" was a crucial substrate for the sympatho-inhibitory component of the baroreceptor reflex: The pressor response to BLCO was significantly reduced following ablation; the pressor response to i.v. NE was significantly enhanced; and small pressor responses elicited by NTS stimulation were potentiated following midline ablation.

Evidence was presented indicating that pressor neurons may reside in classic "depressor areas" of the midline medullary reticular formation and may constitute the site of baroreceptor induced sympatho-inhibition to the blood vessels: Pressor responses were recorded at low stimulus parameters to midline areas; and blood pressure decreased after midline ablation possibly implying the destruction of pressor neurons.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The baroreceptor reflex is probably the most important visceral reflex in the body, yet little is known about its central anatomical organization. In as much as abnormal cardiovascular functions including hypertension and cardiac arrhythmias have been attributed to baroreceptor reflex impairment, it is important to delineate the the medullary neural pathways of the reflex. Also, a clearer understanding of the medullary baroreceptor reflex pathways is important in order to interpret supramedullary modifications of the reflex which are integrated at the medullary level. The purpose of my research was an attempt to elucidate the neural pathways in the medulla involved in the baroreceptor reflex, concentrating on the sympatho-inhibitory component and to ascertain the relative contribution of proposed medullary sites mediating this reflex. The following will be a description of some of the pertinent past research in baroreceptor physiology and anatomy. It may be stated at the onset that the literature is beset with controversies regarding the neuroanatomical pathways of the reflex as well as the contribution and importance of supramedullary structures on cardiovascular function.

Traditionally, the medulla has been regarded as the neural center integrating central cardiovascular control. Dittmar and Owsjannikow in the 1870's were the first to enter into an investigation of the medullary vasomotor center (Bard, 1960). They found that transections of the brain stem from above downward had no effect on blood pressure

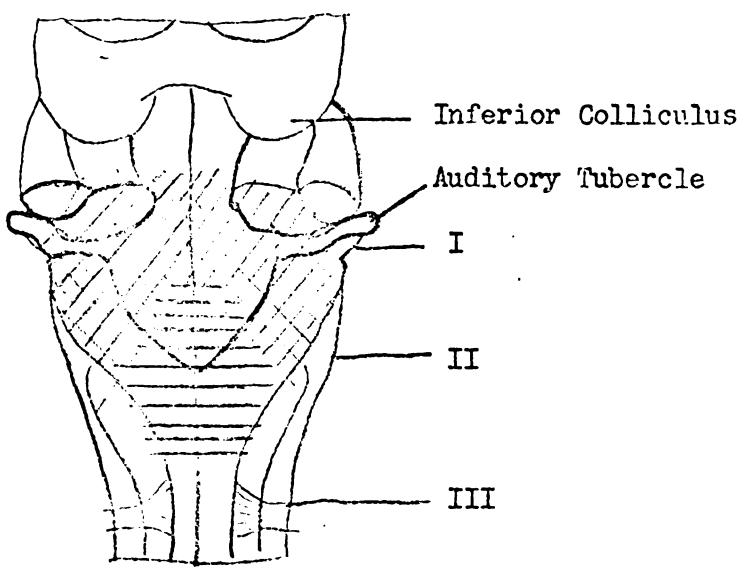
until they reached a pontine level near the rostral border of the trapezoid body. As sections were made more caudally they produced a greater fall in blood pressure. The maximal effect was observed 3-4 mm above the calamus scriptorius. At this point the degree of decrease in blood pressure was the same as that obtained by section of the spinal cord at C-1. These authors also showed that cardiovascular reflexes elicited by stimulation of the sciatic nerve were integrated in this same general ponto-medullary area necessary for maintainance of tonic blood pressure.

Further information regarding the position and nature of the bulbar cardiovascular center was presented by Ranson and Billingsley (1916). They stimulated the floor of the 4th ventricle with a needle electrode and found two discrete reaction points which responded with changes in blood pressure: A pressor center (40 mm Hg. increase in blood pressure) about 1 mm in diameter in the region of the ala cinera to the fovea inferior, and a depressor center (40 mm Hg. decrease in blood pressure) in the area postrema just lateral to the obex. The authors concluded that these points could represent; a) afferent endings of vasomotor neurons, b) receptive nuclei of afferent vasomotor neurons, or c) true vasodilator and vasoconstrictor centers giving origin directly to autonomic efferents.

The next significant research in this area was done by Ranson and Wang (1939). Using the newly designed Horsley-Clark stereotaxic instrument, they were able to explore the depths of the medulla in an attempt to further delineate cardiovascular centers. In general, pressor points were spread diffusely in the dorsolateral reticular

formation, and depressor points in the ventromedial portions of the reticular formation. Thus, these authors showed that the surface areas previously reported by Ranson and Billingsley were just the dorsal extensions of these diffuse areas in the reticular formation.

In 1946, Alexander amplified the concept of cardiovascular centers by presenting a detailed localization of pressor and depressor areas in the pons and medulla. As can be seen from the following figure, the pressor center was found to occupy an extensive region of the lateral reticular formation in the caudal pons and rostral two-thirds of the medulla, while the depressor center includes much of the medial reticular formation in the caudal one-half of the medulla.



Relative distribution of the bulbar pressor and depressor regions: pressor area indicated by crosshatching; depressor area by horizontal ruling. I, II, and III - levels of transection Reproduced from Alexander, 1946.

In attempting to assess the origin of tonic sympathetic nerve activity controlling blood pressure, transections were made as indicated on the

diagram and recordings were taken from the inferior cardiac nerve and cervical sympathetic trunk to indicate changes in cardiac and vasomotor function. Transection as far caudally as the lower one-third of the pons had no significant effect on blood pressure or tonic activity along the sympathetics. Section at I (the level of the auditory tubercle) resulted in a considerable fall in mean blood pressure correlated with a significant reduction in tonic sympathetic activity. This transection removed a significant portion of the rostral pressor region. Section at II (slightly rostral to the obex) produced a maximal fall in blood pressure together with a complete disappearance of activity in the sympathetics. This section, as can be seen from the diagram, removed a large part of the pressor region while leaving a major portion of the depressor region intact. Further section at III (C-1 cord section) raised blood pressure levels from previous low levels and restored some activity in the sympathetic outputs thus implying that the depressor region exhibited tonic activity. That is, the C-1 cord section eliminated a tonic depressor activity descending to the spinal cardiovascular centers from the depressor center in the medulla. This finding, according to Alexander, established the depressor center as a functional entity rather than merely a region through which inhibitory afferents travel to reach the depressor center. The question remains, however, whether this effect depends on the intrinsic activity of a group of midline neurons or is the result of an inflow of impulses from baroreceptor afferents or other sources of a depressor reflex. Alexander (1946) also studied somatic pressor reflexes produced by stimulation of the sciatic nerve. Following removal of portions of

reduction of its discharge rate following proceedures which altered blood pressure levels including i.v. injection of vasoconstrictor and vasodilator agents and bilateral carotid occlusion. These neurons, so defined, could be divided into two groups: Population I- neurons that exhibited an increase in their discharge when arterial pressure rose and a decrease when pressure fell. These neurons ceased firing during bilateral carotid occlusion; Population II- those neurons that behaved in the opposite way, i.e. decreased their firing rate with an increase in blood pressure and increased their firing rate with a decrease in blood pressure. The ratio of type II to type I was about 5 to 1. It was suggested by the author that circulatory control may be achieved by some form of reciprocal innervation between these two systems of neurons similiar to the reciprocity seen in the respiratory centers. On the other hand, it is equally possible that the two systems may operate independently and integrate their activity (inhibitory in the case of type-I neurons and excitatory in the case of type-II) at the spinal cord level. The distribution of type I and type II units did not reveal separate anatomical areas for these units. Type I and type II cardiovascular neurons were found scattered throughout the medulla, intermingled with each other, with the majority of the detectable units within medial structures.

In a study done by Wang and Przybyla (1967) employing similiar techniques as Salmoiraghi, cardiovascular neurons (type II) were located almost exclusively in the periventricular grey and adjacent dorsal reticular formation. These neurons decreased their firing rates from 30-100% following a rise in blood pressure. From this study,

and studies employing lesioning techniques, these authors concluded that the areas responsible for cardiovascular integration are localized in the dorsal lateral portions of the medulla. This group has also demonstrated that supramedullary structures are not essential for cardiovascular integration. This was pointed out by showing that midcollicular decerebration did not abolish the increase in arterial pressure seen after sectioning of the buffer nerves or inhibit the carotid occlusion pressor response. Also, in another study, Chai and Wang (1962) showed that cardioacceleration elicited by dorsal medullary stimulation, bilateral carotid occlusion, or sciatic nerve stimulation was not reduced after midcollicular section.

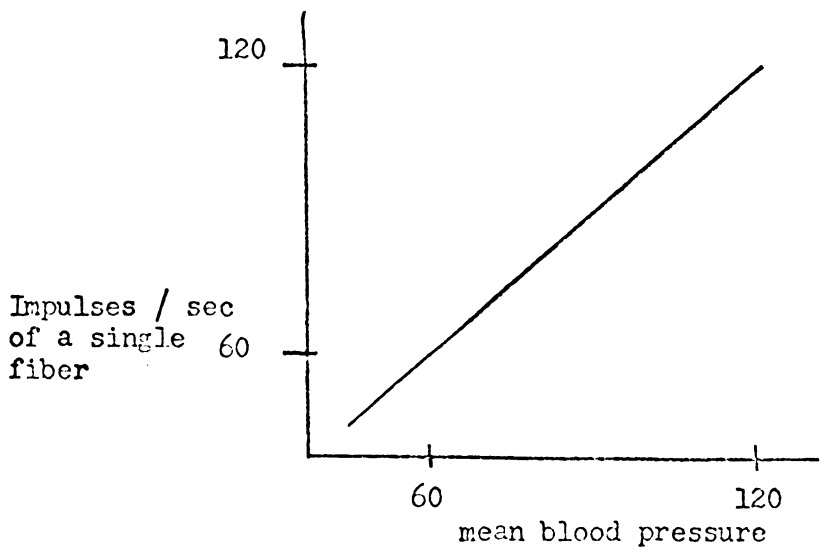
However, Manning (1965) disputes this view that medullary centers located in the dorsal reticular formation maintain vascular tone and form the central synaptic link controlling cardiovascular reflex adjustments. He showed that extensive lesions in the medullary vasomotor area did not significantly alter the cardiovascular reflex adjustments to bilateral carotid occlusion, to stimulation of the sciatic nerve, or to hypothalamic stimulation. In addition the preparations were critically dependent upon supramedullary connections to maintain vascular tone and circulatory reflex adjustments for midcollicular section in the lesioned animal brought about a reduction in blood pressure and a loss of vascular reflex responses. Thus, according to Manning, supramedullary centers exert tonic as well as phasic influences on vascular and cardiac activity independent of the medullary vasomotor area. Evidence from Peiss' laboratory supports this view of Manning(Peiss, 1965). He showed that cardioacceleration produced by

stimulation in the dorsal medulla was eliminated by lesions caudal to the hypothalamus and suggested that the cardioacceleration elicited by stimulation in this area was due to activation of afferent pathways to higher CNS structures. Also, Domino (1968) showed statistically significant decreases in blood pressure following midpontine brainstem transection and suggested that portions of the CNS above the section may be exerting tonic influences on blood pressure. Thus, the question of supramedullary influence on tonic blood pressure and integration of cardiovascular reflexes is still not resolved.

Having discussed the concept of cardiovascular centers and the integration of cardiovascular reflexes in the medulla, I would now like to specifically discuss the baroreceptor reflex including its physiological functions and anatomical representation in the medulla as well as the influence of supramedullary structures on the reflex. The baroreceptor reflex is essentially a negative control system. Baroreceptor nerve endings are found predominately in the carotid sinus region and the arch of the aorta as well as in the right atrium and along the pulmonary arteries. An increase in blood pressure causes mechanical distortion of stretch receptors in these regions and increases tonic nerve activity along the carotid sinus nerve, the aortic depressor nerve in the cat, and the vagus. These fibers terminate in the medulla where they eventually cause inhibition of sympathetic tone to the blood vessels at a medullary site or in the spinal cord to buffer the rise in pressure, and vagal activation to slow the heart. Also, decreases in blood pressure result in a decrease in activity along baroreceptor afferents with a concomitant increase in blood pressure and heart rate due to release of tonic inhibition controlling these

cardiovascular responses. There is also a sympathetic innervation controlling heart rate and cardiac contractility which is modified by baroreceptor afferent input.

In the total population of baroreceptors, there is considerable variation in threshold. At a low but effective pressure, only a minority consisting of the most excitable units contribute to the discharge of afferent nerve impulses. As the arterial pressure rises, more and more units are recruited to augment the impulse bombardment of the baroreceptor afferents. At the same time, those units already active discharge more frequently. The relationship between mean blood pressure and impulse activity has been determined by Bronk and Stella (1934) and is shown in the following graph:



Hence, the rate of impulse discharge is closely related to the level of pressure within the sinus. It was also shown that a pulsatile pressure is a more effective stimulus in exciting baroreceptor discharge than a steady pressure (Neal et al, 1952).

It appears that other physiological functions are mediated or influenced by fibers carried in the baroreceptor afferents. Chemoreceptors are located in the carotid and aortic bodies supplied by afferent fibers of the carotid sinus nerve (CSN) and the vagus. Impulse activity in these fibers is only slight in the anesthesized, spontaneously breathing animal, but is greatly increased by anoxic anoxia, hypercapnia and acidosis. Chemoreceptor stimulation causes both reflex vasoconstirction and hyperpnea. Baust and Heinemann (1967) have suggested that the baroreceptors play a role in the regulation of sleep and wakefullness. They concluded from their studies that the monotonous synchronous inflow from the baroreceptors is in part responsible for the onset and maintainance of synchronized sleep. A related study by Bonvallet (1954) showed that EEG activity is influenced by stimulation of carotid sinus afferents; increased pressure resulted in EEG synchronization via a reflex inhibition of ascending reticular neurons. Bonvallet also showed in this study that interruption of the baroreceptor pathway at the nucleus of the tractus solitarius (NTS) intensifies the arousal pattern in the EEG induced by somatic or auditory stimulation of the reticular activating system. Zanchetti et al (1960) demonstrated that diencephalic centers responsible for sham rage are under a tonic inhibitory influence from the carotid sinus and aortic pressoceptors. They showed that de-afferentation of the pressoceptors results in an increase of somatic and autonomic outbursts, while increasing intrasinusal pressure blocked spontaneously occuring outbursts of sham rage. Also in acute decorticate cats, bilateral carotid occlusion induced outbursts of sham

rage which were abolished by carotid sinus nerve section. In addition to the afore mentioned activities, baroreceptor afferent impulses also modify the spinal somatic reflexes as well as influence ADH secretion (Share, 1965, Rothballer, 1963). From these studies, then, it is evident that blood pressure changes mediated by baroreceptor afferents result in widespread and diverse physiological modifications which might conceivably alter the direct cardiovascular compensatory mechanisms mediated by the baroreceptor reflex.

Turning now to the neural representation of the baroreceptor reflex in the medulla, I would like to discuss papers which attempt to elucidate the medullary neural pathways as well as mention some studies demonstrating supramedullary modifications of the reflex. As will be shown, there are huge gaps in our knowledge of the interactions between the various inputs and the integrative centers of the medulla concerned with cardiovascular function. It has been proposed from evidence derived from degeneration studies that the principal site of termination of baroreceptor afferents is in the middle third of the nucleus of the tractus solitarius (NTS) near the obex (Cottle, 1964, Kerr, 1962). Descrepencies exist as to where second order neurons project mediating sympatho-inhibition and vagal activation, and if, and to what extent, classic "depressor centers" of the midline reticular formation participate in the baroreceptor reflex.

Morest (1967) failed to show connections from NTS to the midline based on axonal degeneration studies following lesions in the posterior one-half of NTS. The most significant projections of NTS were traced to the dorsal lateral reticular formation of the medulla. This area

has been implicated as the primary integrating center for cardiovascular reflexes as well as the most prominent locus of tonic cardiovascular neurons (Chai and Wang, 1968). Projections were also traced by Morest to nucleus ambiguous after NTS lesions. This finding supports the contention of Gunn et al (1968) who postulated that nucleus ambiguous is the site of origin of vagal cardiomotor efferents.

Advances in electrophysiological recording techniques have resulted in a more precise exploration of medullary pathways involved in cardiovascular reflex mechinisms. Sampson and Biscoe (1968) recorded electrical potentials evoked in the brain stem by stimulation of the carotid sinus nerve. Based on onset latencies, they showed monosynaptic connections to NTS which relayed monosynaptically to the ventromedial reticular formation of the medulla. Recording extracellularly, they detected positive potentials (indicating a decrease in excitability) in the following medial areas: 0.5-1.8 mm from the midline; 2 to 5 mm from the ventral surface; and from -10 to - 13 mm (Horsley-Clark coordinants) in the A-P plane. In several trials, when the recording electrode penetrated one of the cells in this area, a hyperpolarizing IPSP was detected with the same time course as the extracellular positivity. Histological sections revealed involvement of the following medial nuclear groups: Subnucleus ventralis reticularis oblongata; nucleus reticularis gigantocellularis; and possibly the medial nuclei of the raphé. They also concluded from their study that secondary fibers may be crossing the midline at many levels in the reticular formation based on potentials recorded contralaterally from A-8 to A-16.

Crill and Reis (1968) observed a similiar distribution of baroreceptor afferents. They explored the medulla with stimulating electrodes and recorded antidromic potentials in the carotid sinus nerve and the aortic depressor nerve in the cat. Potentials were recorded following stimulation in NTS as well as in the medial reticular formation including paramedian reticular nucleus (PMN), nucleus gigantocellularis, and nucleus medullae oblongatae centralis. They also recorded potentials from stimulation of the cuneate complex suggesting that tonic baroreceptor input may be responsible for the spontaneous activity recorded at this site by many investigators.

Further evidence that the midline is involved in the baroreceptor pathway is presented by Humphrey (1967). He applied single shock stimulation to the ipsilateral carotid sinus nerve and recorded potentials within two relatively distinct zones of the medial reticular fromation. One region consisted of a ventral midline strip about 1 mm in dorsal-ventral length bounded ventrally by the internal arcuate fibers. The second zone consisted of an area approximately 1 mm from the midline which extended ventrally from 2 to 4 mm beneath the floor of the fourth ventricle. In addition to the medial regions, potentials were observed in the dorsal reticular formation, ventral to the nuclei of the 9th, 10th, and 12th cranial nerves (area of NTS). No evoked potentials were recorded from the "pressor regions" of the lateral reticular formation. Since the onset latencies of these potentials indicated polysynaptic pathways, Humphrey stimulated points in NTS that had responded to carotid sinus nerve stimulation and recorded potentials in the same areas as shown previously. Thus, the polysynaptic

nature of the pathway was confirmed, with NTS a relay point in these pathways between sinus nerve afferents and the depressor regions of the medullary reticular formation. As mentioned previously, stimulation of baroreceptors is known to produce such diverse effects as respiratory changes, alteration of somatic reflexes, EEG changes, etc, so these pathways delineated by Humphrey may be mediating such effects rather than hypotensive effects.

Recent electrophysiological evidence of Miura and Reis (1969) indicates that baroreceptor afferents terminate in RVN as well as NTS. This dual termination in the medulla was suggested by the short latency, monosynaptic responses recorded at these sites by stimulation of the carotid sinus nerve. This indicated a direct pathway whereby carotid sinus nerve activity could reach the medial reticular formation without synapsing in NTS. It is interesting to note that RVN may be involved in the baroreceptor pathway. Studies have shown that its integrity appears necessary for sustaining the depressor responses elicited by electrical stimulation of forebrain (Lofving, 1961), and muscle nerves (Heymens and Neil,1958) as well as some cardiovascular responses elicited from the cerebellum (Miura and Reis, 1968). Thus, supramedullary modulating effects on baroreceptor activity may be integrated at this site.

Additional evidence implicating the midline depressor area in the baroreceptor reflex was presented by Chai and Wang (1968). Following ablation of discrete areas in the midline, they showed that the pressor response to bilateral carotid occlusion (BLCO) was significantly reduced. They also showed that ablation of the periventricular grey

or the "pressor area" in the dorsal lateral reticular formation reduced the BICO response as well as the pressor response to stimulation of the sciatic nerve. From these results they postulated that afferent pathways of the baroreceptor reflex pass through the midline depressor region and on to the lateral pressor areas.

From the foregoing, it appears that NTS is the first relay station in the baroreceptor reflex pathway. As stated previously, the descrepensies exist as to the projections from this area to the site of sympatho-inhibition of tonic pressor neurons which may be in the medulla or the spinal cord (Lim et al, 1938, Seller and Illert, 1968, Alexander, 1946). Termination of projections from NTS in the depressor areas has been proposed by several authors previously mentioned. This is compatable with the anatomical description of reticular neurons shown by Brodal (1957) and the Scheibels' (1957). Their studies demonstrate that reticular neurons located in the medial regions of the brain stem characteristically have long axons which ascend or descend or both thus serving as effector neurons whereas neurons in the lateral reticular fromation send their axons medially and are, by nature, association or sensory neurons. This important anatomical description of reticular neurons seems to have been neglected by many physiologists working in this area. Since most of the "pressor area" is situated outside those regions which give off reticulospinal fibers, one must question the proposals that show the final synaptic link in the medulla at the lateral reticular fromation. How do the effector axons of these neurons reach the intermediolateral columns of the spinal cord which contain the cells of the final common pathway to the vessels? One should

also question the efficiency of a meandering pathway that goes from lateral areas to the midline and back to the pressor regions in the lateral reticular formation such as proposed by Chai and Wang (1968). Teleologically, one would not expect such an arrangement. The most efficient pathway compatable with anatomical and physiological evidence would be from NTS to the midline and down to the spinal cord placing the site of sympatho-inhibition in the cord or perhaps in the medullary "depressor region"- a possibility which to my knowledge has never been proposed. I will be presenting some evidence that is compatable with this idea.

Before concluding a discussion of the neural pathways, I would like to briefly describe some studies demonstrating supramedullary modifications of the baroreceptor reflex. Knowledge of these mechanisms may aid in elucidating the pathways and neural structures involved in the medullary representation of the reflex. Since there have been many studies recently demonstrating supramedullary modifications of the baroreceptor reflex, I will limit my discussion to those which have implicated a specific medullary termination site along the baroreceptor pathway. Hockman (1969) has shown that stimulation of the fastigial nucleus in the cerebellum in spinal cats inhibits the bradycardia produced by stimulation of the carotid sinus nerve. The degree of inhibition depended on the amount of impulse traffic in the baroreceptor afferents. Since there is a prominant efferent fastigiobulbar pathway to IRN and since carotid sinus nerve afferents have been shown to terminate around this nucleus, it was postulated that cerebellar modification (inhibition) of the baroreceptor reflex occurs

at this site.

Carlaresu and Henry (1970) have implicated nucleus intercalatus (NIc) as a site of integration of cardiovascular reflexes in the medulla. Stimulation of this area (parahypoglossal) produces an increase in blood pressure and heart rate. NIc receives projections from the hypothalamus as well as from NTS (Morest, 1967). Thus, this site may be part of a descending hypothalamic pathway that acts to inhibit compensatory baroreceptor reflexes in order to maintain an adequate blood flow to critical tissues in times of stress and exertion.

Smith and Nathen (1966) have shown that stimulation of medial portions of the inferior olivary nucleus can inhibit the bradycardia and the depressor effect of carotid sinus stretch and suggested that this area may serve to integrate supramedullary influences from the midbrain and hypothalamus on baroreceptor compensatory reflexes.

From these studies, it appears clear that suprabulbar influences on cardiovascular functions are not exerted by modulating the excitability of the " primary cardiovascular neurons " located in the medullary reticular formation but rather act on discrete loci presumably in the baroreceptor reflex pathway which are distinct from the tonic vasomotor neurons. Also, it is evident that the reflex is quite labile and appears to be overidden by neural centers above the medulla during environmental stress including emotions, exercise and perhaps certain pathological conditions such as hypertension.

In an attempt to characterize the cardiovascular responses elicited from proposed medullary sites in the baroreceptor reflex, stimulation techniques were employed. The following nuclear sites were investigated:

NTS; DMN; and the inferior olivary nucleus. The participation and contribution of sympathetic and parasympathetic components of the reflex were evaluated by obtaining cardiovascular responses before and after vagotomy. The second phase of the experiments involved ablation of various medullary sites in order to ascertain the importance and relative contribution of the lesioned areas to the baroreceptor reflex.

METHODS

Cats weighing 2.0 to 3.5 kg. were used for these experiments. They were anesthesized with Dial-Urethane (0.7 cc/kg.). A heating pad was employed to maintain rectal temperature at 37° C. Tracheal cannulation was performed on all cats so that artificial ventilation could be administered when necessary. Blood pressure was recorded from a femoral artery with a Statham P-23 series pressure transducer connected to a Grass polygraph. Beat to beat changes in heart rate were recorded using a Grass cardiotachometer which triggers to each femoral pulse wave. Drugs were injected into the left femoral vein. The carotid arteries were disected free in the neck region and separated by a loose ligature to insure easy accessibility for occlusion proceedures. Small bulldog clamps were used for bilateral carotid occlusion. The duration of the occlusion was limited to 30 seconds in order to minimize cerebral anoxia which may distort the cardiovascular responses measured. In several experiments, the vagal trunk was ligated so that vagotomy could be performed to ascertain the relative contributions of the parasympathetic components of cardiovascular function.

Brain Stimulation

Stimuli were applied before and after vagotomy to selected areas of the medulla to characterize the cardiovascular responses of proposed sites in the baroreceptor reflex and to evaluate the sympathetic and parasympathetic contributions of these responses. Stimuli were delivered by means of a square wave stimulator the output of which was passed through a stimulus isolation unit to insulated stainless steel bipolar or concentric electrodes. The cats were placed in a stereotaxic frame.

Portions of the occipital and parietal bones were removed to permit electrode placement. Partial corebellectomy was performed by suction to allow a clear visualization of the dorsal medulla.

NTS stimulation: With the vagi intact, the middle third of NTS just rostral to the obex was explored until a maximal bradycardia was elicited. This point was used throughout the experiment and marked by a leisoning instrument (1.5 ma) for subsequent histological examination. Voltage-response curves were plotted varying the voltage from 2.5 to 10 V and keeping the duration (0.1 ms) and frequency (20 cps) constant.

PMN stimulation: The electrodes were carefully positioned according to the coordinants of Berman (1968) and a locus in PMN was selected which yielded a large depressor response (approximately P-13 mm, 0.5 mm from the midline, and 2 ± 2.5 mm from the dorsal surface). Voltage-response curves were obtained (2.5 to 15 V), keeping the duration (0.5 ms) and frequency (50 cps) constant.

Inferior olivary nucleus stimulation: The electrode placement was determined by exploring the inferior olive and locating large depressor responses. The approximate coordinants were as follows: P-11 mm, 0.5 mm from the midline, and 3.5 to 4.5 mm from the dorsal surface. Voltages varied from 2.5 to 10 V. ; 0.5 ms and 50 cps were kept constant.

Ablations

Ablations were made bilaterally in NTS, PMN, the dorsal lateral reticular formation (DLRF) of the medulla, and the extent of the midline depressor area with a Stoelting Lesion Producing Device.

NTS ablation: Cardiovascular responses were recorded following unilateral and bilateral NTS ablation. A 15 minute stabiligation

period ensued between lesioning and recording responses.

NMN ablation: The lesioning electrode was positioned so that the ablations would include the site of monosynaptic termination of CSN afferents described by Muira and Reis. Lesions were made bilaterally 0.75 mm rostral and 0.75 mm caudal to the obex. The lateral coordinant was 0.75 mm from the midline; the dorsal-ventral coordinant was 2.5 mm from the dorsal surface. Responses were recorded 15 minutes after lesion. Extended lesions were made in most experiments in 1 mm steps in the rostral plane to include the anterior portions of NMN. The optimal lesioning current was 3 ma/15 sec.

DLRF ablation: Lesions were made bilaterally in the DLRF including an area from the obex to 6 mm above the obex. The electrodes were placed 3 mm lateral to the midline and 2.5 mm from the dorsal surface. Responses were recorded after the total extent of the lesion. Lesioning parameters were 5 to 10 ma/20 sec.

Midline depressor area ablation: The lesions were performed in the following manner in an attempt to destroy most of the midline depressor area: Lesion #1; 0.5 and 1.5 mm above the obex, bilaterally, 2.5 and 4.0 mm below the dorsal surface of the medulla, and 0.75 mm from the midline. Therefore a total of 8 lesion points. Lesion #2; 2.5 and 3.5 mm above the obex with the same dorsal-ventral and medio-lateral coordinants. Lesion #3; 4.5 mm above the obex with the same dorsal-ventral and medio-lateral coordinants. Cardiovascular responses were recorded 15 minutes after each set of 8 lesions. The lesioning parameters were 3 ma/15 sec.

Baroreceptor Activation

Activity in baroreceptor reflex pathways was modified in the following manner: Bilateral carotid occlusion; i.v. injection of NE; and stimulation of nuclear sites proposed to be in the pathway. When the carotids are clamped bilaterally distal to the stretch receptors in the carotid sinus region, a pronounced reflex rise in blood pressure would ensue because there would be few impulses along baroreceptor afferents to inhibit sympathetic vascular tone. That is, the tonic sympatho-inhibitory input would be reduced. Injection of NE raises blood pressure and increases baroreceptor input. If the ablations interrupted the reflex pathways, one would expect the NE pressor response to be enhanced since the reflex mechanisms could not compensate for the rise in pressure. The third method employed to alter baroreceptor reflex activity involved electrical activation of nuclear sites proposed in the pathway. By testing the cardiovascular responses elicited by stimulation of these sites before and after ablation, one can ascertain the relative importance of the ablated site to the baroreceptor pathway.

Histology

The extent of the lesions as well as the placement of stimulating electrodes were elucidated by gross section after fixation in 10% buffered formalin. Histological verification was attempted on selected brains from each set of experiments employing the following proceedures: 1) fixation in formalin for several days. 2) dehydration in various concentrations of alcohol and xylene. 3) embedded in paraffin. 4) sectioned in 20 micron thickness and 5) stained with cresyl violet

for cells and luxol fast blue for fiber tracts.

Analysis of Data

All values are expressed as the mean ± SE. Statistical analysis was performed with the Student t test for paired and grouped data. A "P" value of <0.05 was considered to indicate statistical significance. Also, a coefficient of correlation was obtained in one group of experiments employing the method proposed by Bravais and Pearson (Lewis, 1966).

RESULTS

In the first set of experiments, NTS, the proposed site of termination of baroreceptor afferents, was stimulated before and after vagotomy in order to characterize the cardiovascular responses and to ascertain the relative contributions of parasympathetic and sympathetic components mediating these responses. Figure 1 shows typical responses to NTS stimulation. It is evident that NTS mediates both sympatho-inhibition to the vessels as well as vagal activation. The responses seen following vagotomy indicate that the bradycardia resulting from NTS stimulation is predominantly mediated by parasympathetic fibers, although there appears to be a small sympatho-inhibitory contribution as indicated by the residual bradycardia following vagotomy. The fact that the depressor response is essentially unchanged following vagotomy suggests that this is a sympatho-inhibitory response independant of vagal influence. Figure 2 summarizes the results of NTS stimulation on heart rate responses in 7 cats. Here again it is clear that vagotomy substantially reduces the bradycardia evoked by NTS stimulation. Also, it appears that the vagal pathway eminating from the stimulated area in NTS is uncrossed as ipsilateral vagotomy reduces heart rate responses to about the same level seen after bilateral vagotomy. The next figure (figure 3) represents the depressor responses resulting from NTS stimulation at various voltages. It appears that the vagus does not contribute to the depressor responses as the responses seen after vagotomy are similar at most voltage parameters.

In the next series of experiments, paramedian reticular nucleus (PRN) was stimulated. The purpose of this was to ascertain the

cardiovascular responses as this area was proposed by Miura and Reis (1969) to be a monosynaptic termination site of CSN afferents. Also, the degree of vagal participation in the responses could be evaluated following bilateral vagotomy. Figure 4 summarizes the results of 5 experiments depicting the depressor responses to PMN stimulation at varying voltages. It is evident that there is a large depressor component (60 mm Hg.) which is still present after vagotomy indicating a pure sympatho-inhibitory response. Figure 5 shows the concomitant fall in heart rate during PMN stimulation. The small bradycardia evoked by electrical activation of PMN is evident after vagotomy implying that the decrease in heart rate is mediated by sympatho-inhibitory fibers to the heart. Figure 6 represents typical cardiovascular responses to PMN stimulation. The important point to be noted is that the large depressor responses are converted to small pressor responses at low voltage intensities. This unexpected observation was seen in several experiments and may suggest that pressor neurons reside in the classic "depressor regions" of the medulla. Further explanations will be offered in the discussion section.

The next series of experiments involved electrical activation of the inferior olivary nucleus. This nuclear mass is located in the classic "depressor regions" of the medial reticular formation and has been implicated as a possible integrative center for supramedullary influences (midbrain, cerebellum and hypothalamus) on the baroreceptor reflex. Figure 7 represents graphically the results of the effect of inferior olivary stimulation on blood pressure in 3 cats. Depressor responses are similar before and after vagotomy. Table 1 summarizes

the cardiovascular responses evoked by stimulation of this area. It is noted that heart rate is hardly influenced by stimulation of the inferior olive.

The next group of experiments employed ablation techniques to interrupt proposed baroreceptor reflex pathway sites. These experiments were done on vagotomized cats in order to evaluate the sympathoinhibitory component of the baroreceptor reflex. Activity in baroreceptor reflex pathways was modified in the following manner: Bilateral carotid occlusion; i.v. injection of NE; and stimulation of nuclear sites proposed to be in the pathway. By employing these techniques, one is able to directly alter baroreceptor reflex activity and evaluate the changes in activity which would occur if ablations interrupted part of the neural substrate of the reflex.

The first series of ablations was done on NTS. After control responses were obtained, NTS was lesioned on the right side. Responses were retested after a 15 minute stabilization interval. NTS was then lesioned on the left side and the responses were again tested. Table 2 summarizes the data obtained from 6 cats. Following ablation mean blood pressure was increased, however not significantly. The bilateral carotid occlusion response (BLCO), the most sensitive indicator of baroreceptor reflex activity, was significantly reduced after unilateral NTS ablation. Further reduction of the response occurred after bilateral ablation: It was reduced to 45% of the control value. Pressor responses to NE increased slightly after bilateral NTS ablation. The interaction studies done with RMN stimulation revealed that the depressor response seen in control was augmented following bilateral

ablation of NTS. Figure 8 shows the typical reductions of the pressor response to BLCO following unilateral and bilateral NTS lesion. This reduction was seen in every animal tested.

In order to evaluate the importance of PVN in the baroreceptor pathway, ablations were performed at this site in 6 cats. The lesioning electrode was positioned to include the area of PVN where Miura and Reis detected monosynaptic terminations of CSN afferents. Table 3 summarizes the results. Of interest is the observation that the BLCO response was not significantly reduced; reduction was only to 90% of control value. Also, blood pressure decreased following ablation which would not be expected if PVN was involved in the reflex pathway.

The next group of ablations involved the dorsal lateral reticular formation of the medulla. This area, as mentioned previously, was implicated by Chai and Wang to represent the site of tonic pressor output to the vessels as well as the central integrating locus for cardiovascular reflexes. Table 4 lists the results from 3 cats. It is evident from this data that central cardiovascular function is markedly depressed. However, gross inspection of the lesioned site revealed massive destruction probably extending to interrupt afferent fibers of the reflex. Also, it was noted that extensive bleeding occurred. Therefore, it is doubtful that these results represent an accurate assessment of the role of the dorsal lateral reticular formation in baroreceptor function. Reevaluation of this area is planned in the future with the purpose of producing more discrete lesions.

Since PVN ablation did not significantly alter baroreceptor reflex

function and because midline depressor areas have been implicated in the reflex pathway, it was decided to extend midline ablations to include an area from the obex to about 5 mm above the obex. The lesioning sites were described in the methods section. Table 5 lists the data from 10 cats. A significant reduction of the BLCO response was noted after the second set of lesions implying that the midline area encompassed by the lesion was part of the baroreceptor pathway. However, examination of the data revealed that when mean blood pressure increased after lesion (cat #7), the BLCO response also was increased from control; and when resting blood pressure decreased, the BLCO response decreased. Figure 9 shows this correlation. The value obtained for a coefficient of correlation was 0.722 which was significant at the 5% level. Table 5 also shows that the response to i.v. NE is significantly potentiated after two sets of lesions. It is also noted that heart rate increased progressively following lesions. Figure 10 depicts the typical reduction of the pressor response to BLCO following the first and second set of lesions in which resting blood pressure had decreased. The bottom panel represents the potentiation of the NE pressor response in the same animal after lesioning. The following figure (fig. 11) shows an example of potentiation of the BLCO response after ablation in which blood pressure has increased from control values. Another finding which may implicate the midline in the baroreceptor pathway is illustrated by figure 12. It is evident that a small pressor response elicited by NTS stimulation becomes progressively potentiated as more of the midline is ablated. Table 6 summarizes the data comparing blood pressure responses to NTS stimulation before and after lesion.

Figure 1

The effect of vagotomy on the cardiovascular responses elicited by NTS stimulation. Panel B shows the blood pressure and heart rate responses to NTS stimulation with the vagi intact. Panel A shows the cardiovascular responses after bilateral vagotomy. The downward deflections of the time base indicate the duration of NTS stimulation. Parameters of stimulation are: 10 V., 0.1 m.s., and 20 c.p.s. BP=blood pressure in mm Hg. HR=heart rate in beats/min.

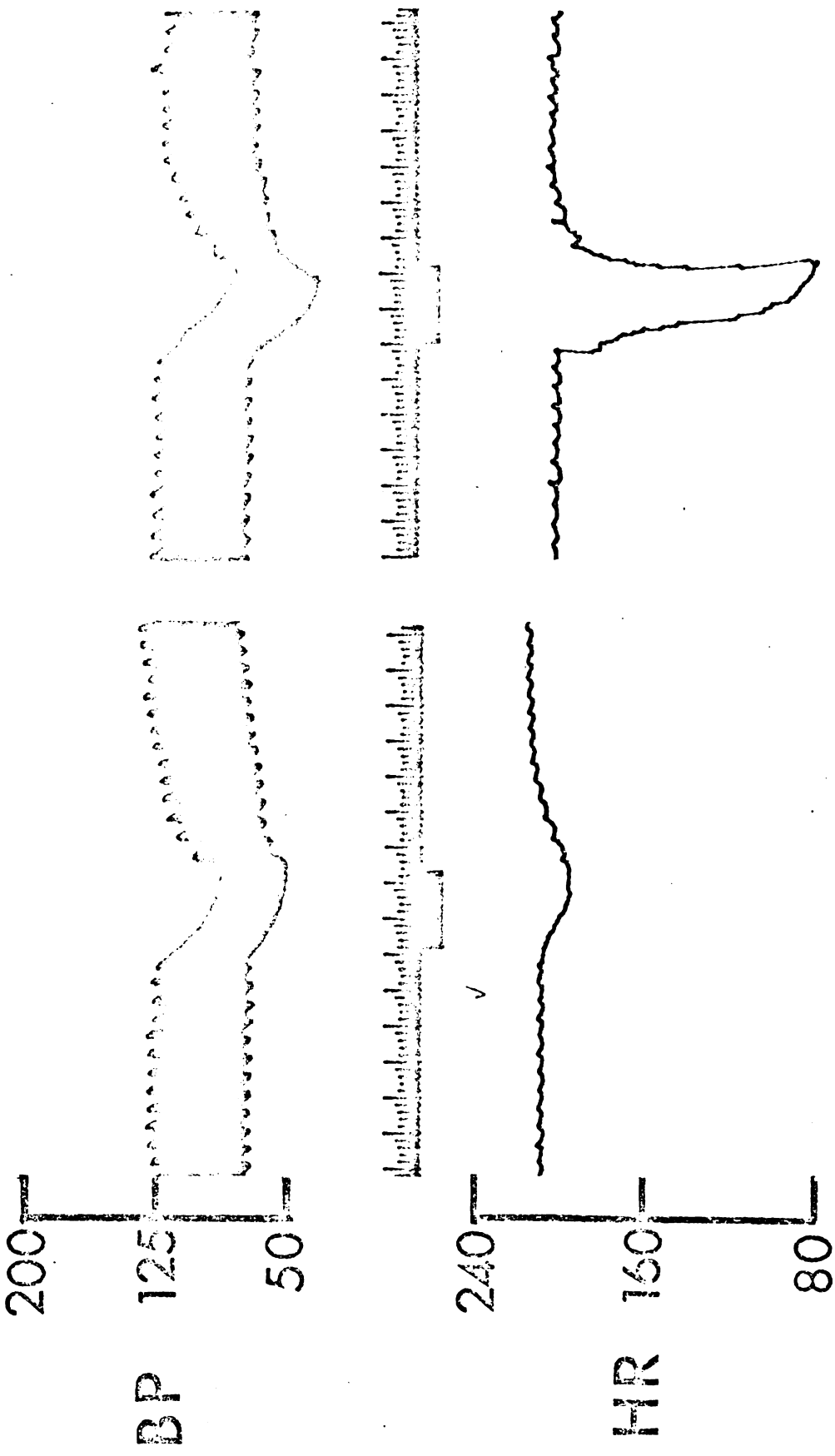

A
B
BP
200
125
50
HR
240
160
80

Figure 2

Voltage-Response Curve showing the effect of vagotomy on heart rate responses elicited by NTS stimulation at varying voltage intensities. Each point represents the mean and the standard error of 7 experiments. The stars represent heart rate responses with both vagi intact; open circles represent heart rate responses following ipsilateral vagotomy; triangles represent heart rate responses after bilateral vagotomy. The voltage intensities were varied from 2.5 to 10 V. The duration (0.1 ms) and frequency (20 cps) were kept constant.

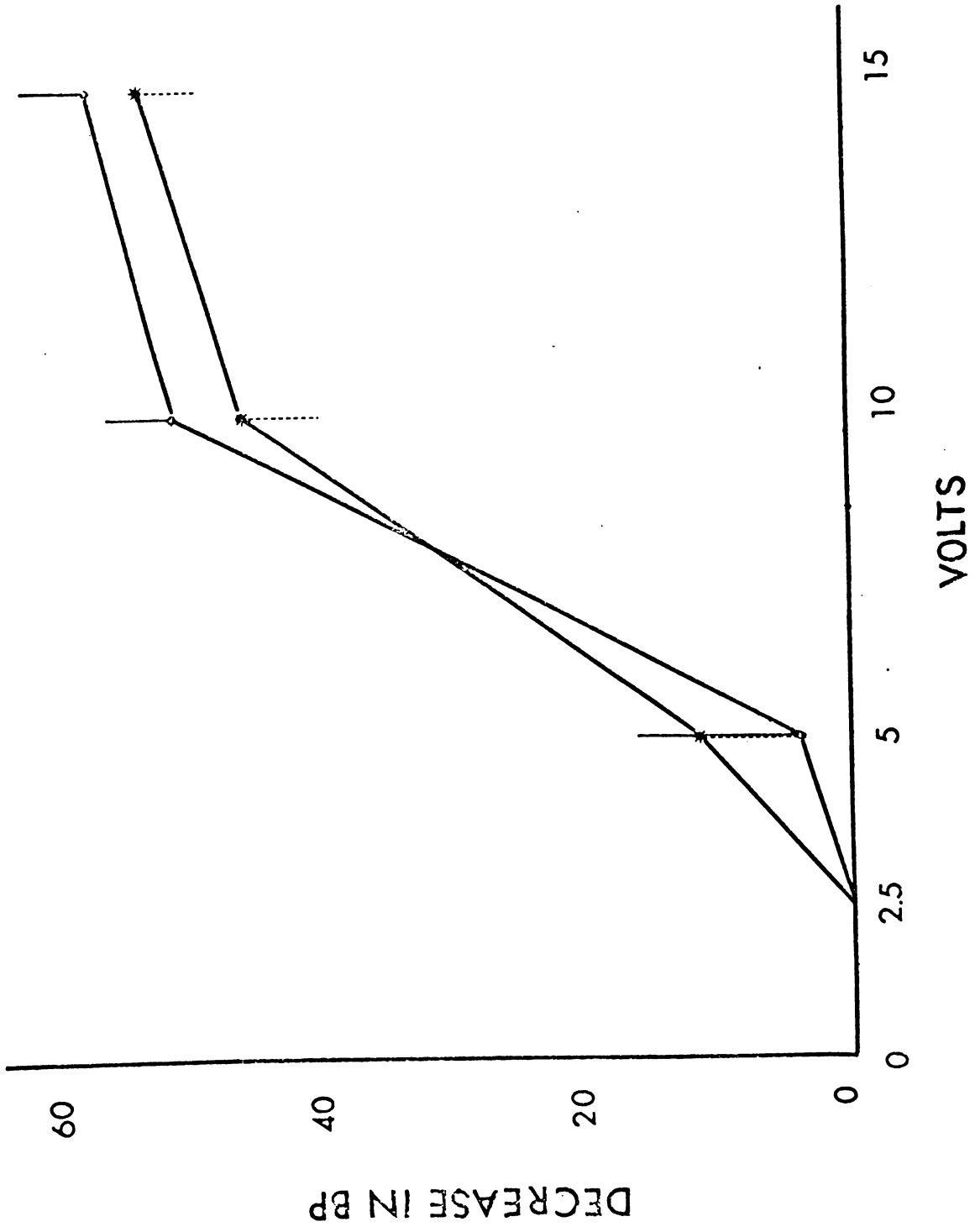

DECREASE IN BP
60
40
20
0
0
2.5
5
10
15
VOLTS

Figure 5

Voltage-Response Curve showing the effect of vagotomy on the heart rate responses evoked by PMN stimulation. The points represent the mean and standard error of 5 experiments. The open circles represent the heart rate responses evoked by PMN stimulation with the vagi intact; stars represent the heart responses after bilateral vagotomy. The voltage intensities were varied from 2.5 to 15 V. The duration (0.5ms) and frequency of stimulation (50 cps) were kept constant.

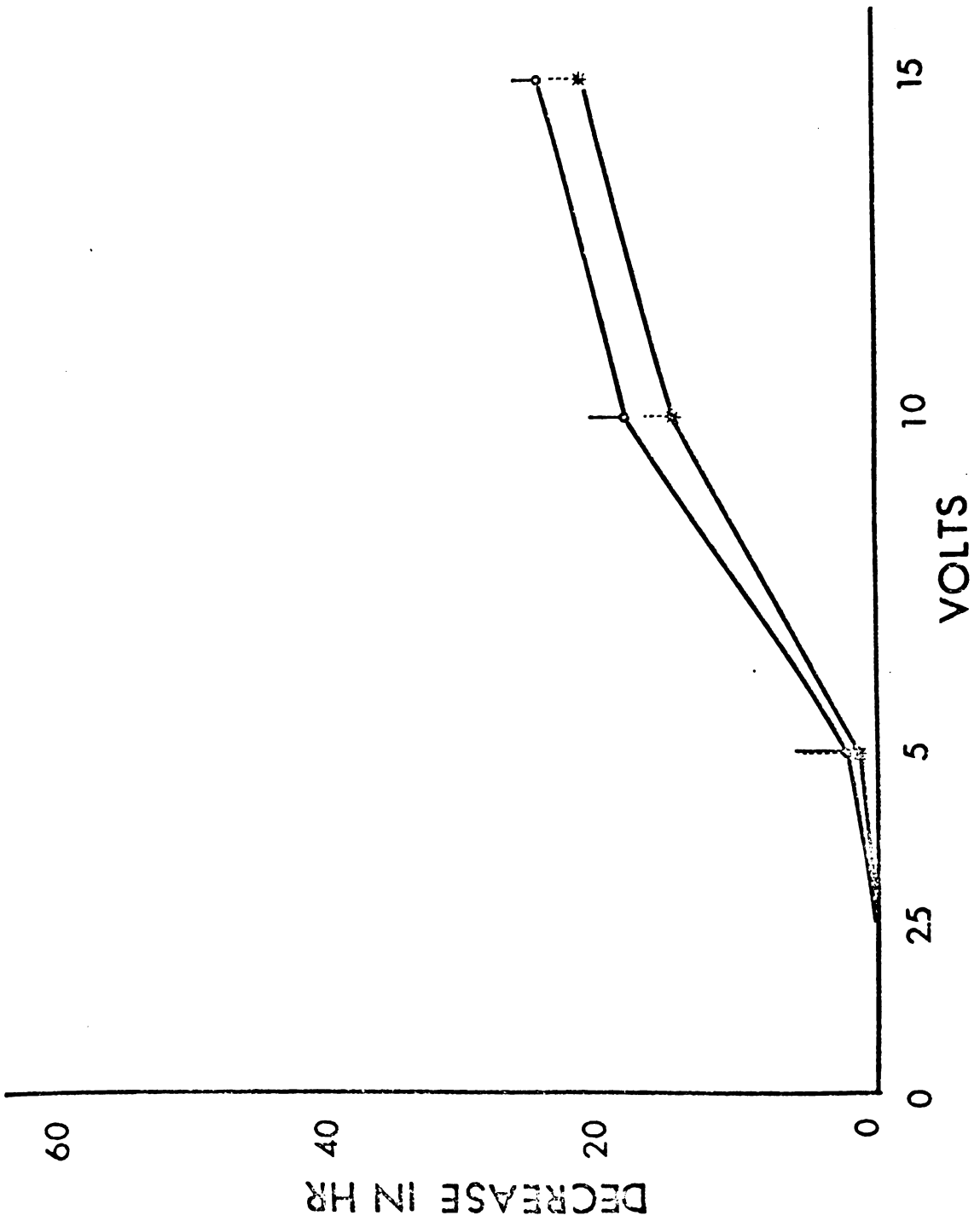

DECREASE IN HR
60
40
20
0
0
25
5
10
15
VOLTS

Figure 6

The effect of varying voltage intensities on the cardiovascular responses evoked by stimulation of PAG. The top two panels represent the cardiovascular responses elicited at varying stimulus intensities to PAG with the vagi intact. The bottom two panels represent the responses after bilateral vagotomy. The downward deflections of the time base indicate the duration of PAG stimulation (10 sec). The headings at the top are the various stimulating intensities. The duration (0.5 ms) and frequency of stimulation (50 cps) were kept constant.

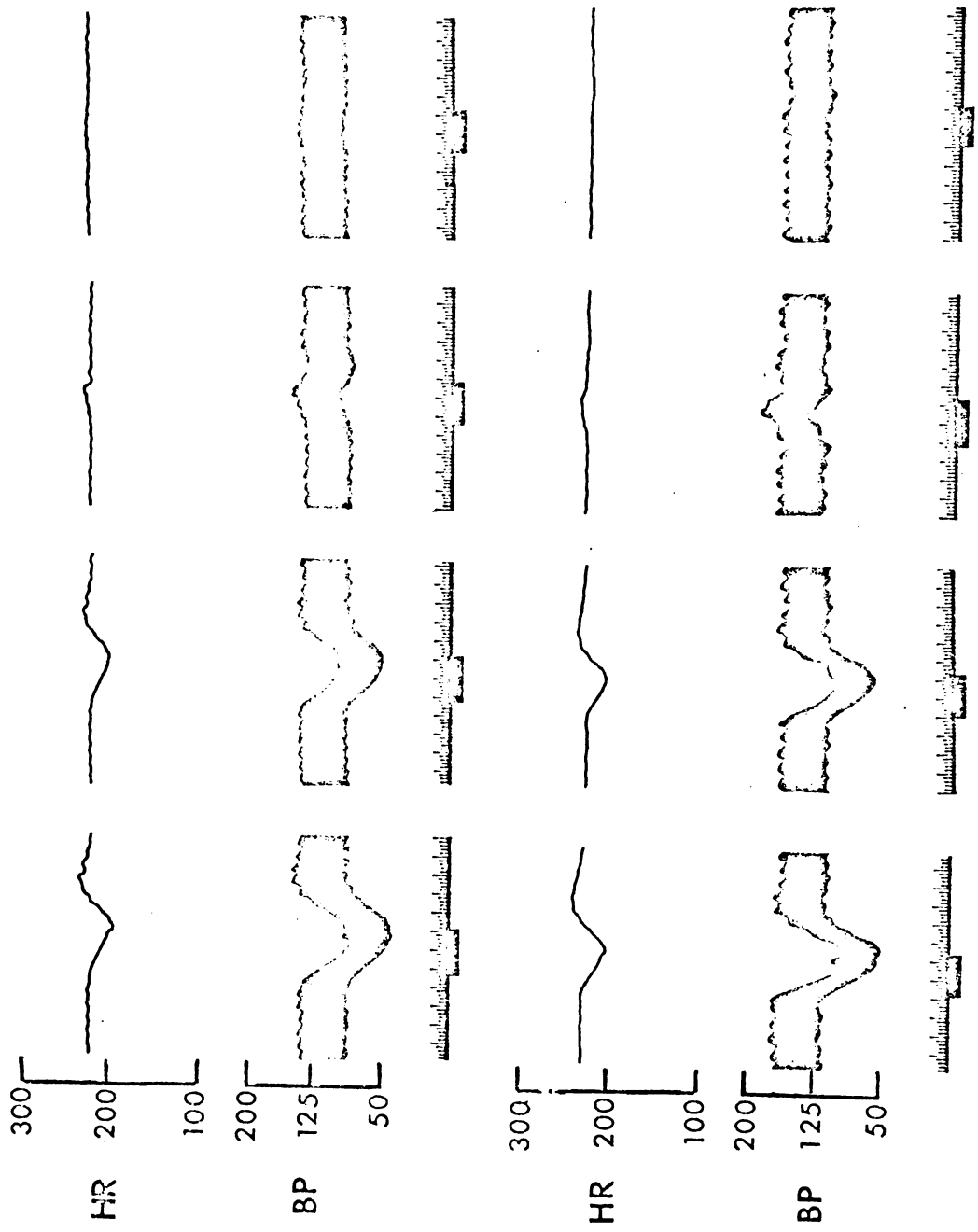

15V
10V
5V
2.5V
HR
BP
300
200
100
200
125
50

Figure 7

Voltage-Response Curve showing the effect of vagotomy on the depressor responses elicited by inferior olive stimulation. Each point represents the mean and standard error of 3 experiments. The stars represent the depressor responses elicited by stimulation of the inferior olive with the vagi intact. The circles represent the responses elicited by stimulation of the same point in the inferior olive after bilateral vagotomy. The voltages were varied from 2.5 to 10 V. The duration (0.5ms) and frequency of stimulation (50 cps) were kept constant.

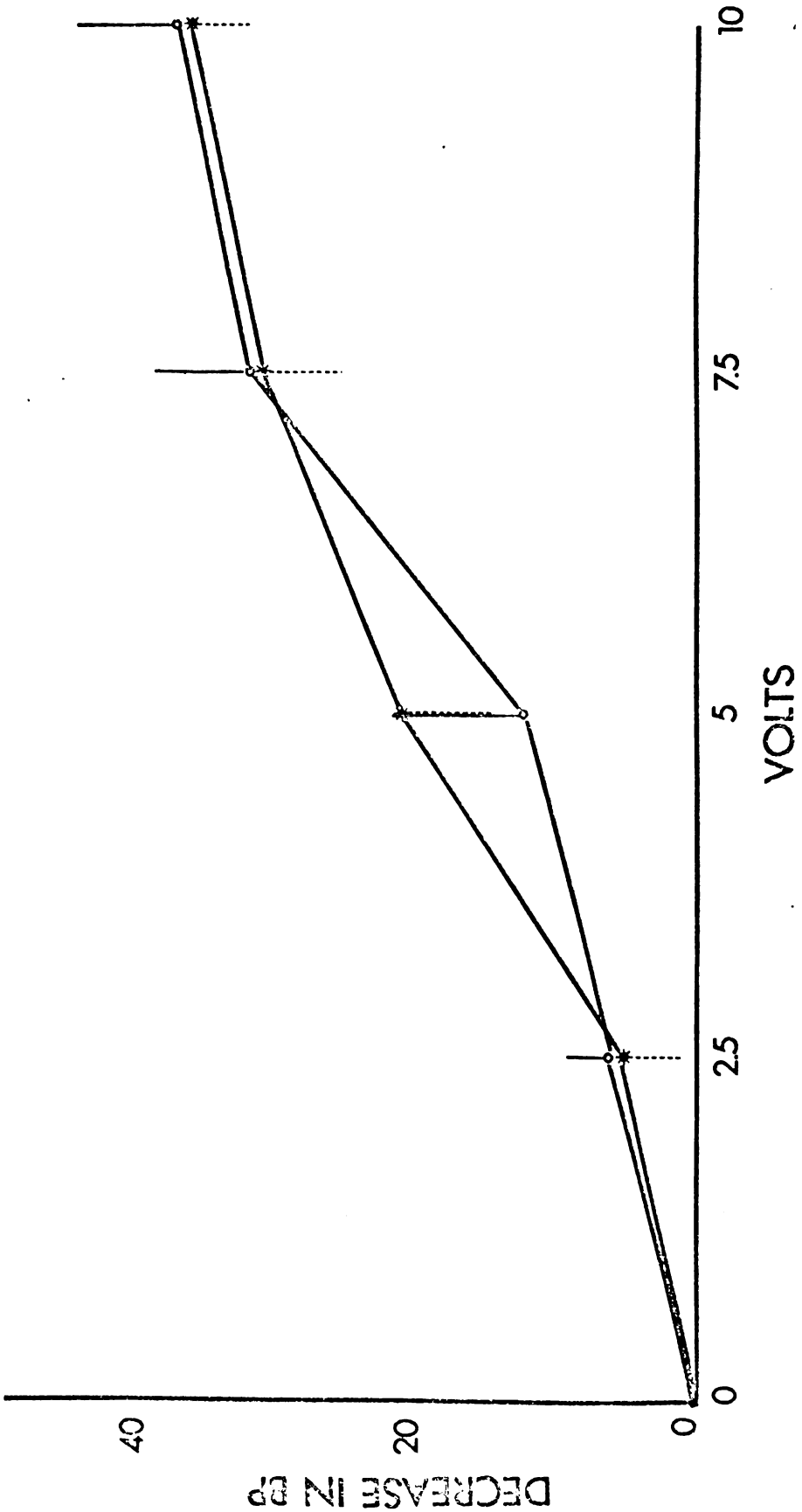

DECREASE IN EP
40
20
0
0
2.5
5
7.5
10
VOLTS

Table 1

The effect of vagotomy on cardiovascular responses elicited by inferior olive stimulation. The numbers represent the mean and standard errors of 3 experiments. The voltage intensities were varied from 2.5 to 10 V. The duration and frequency of stimulation were kept constant: 0.5 ms and 50 cps.

	CONTROL		VAGOTOMIZED	
	Δ MEAN B.P.	Δ H.R.	Δ MEAN B.P.	Δ H.R.
10 V	-36.67±4.41	+1.33±5.67	-37.67±7.22	-4.67±3.33
7.5 V	-31.67±6.01	0.00	-32.67±6.74	-2.00±3.05
5.0 V	-21.67±7.26	+0.67±0.67	-12.67±9.33	+0.60±3.48
2.5 V	-5,67±5.67	0.00	-6.00±3.05	0.00

Table 2

The effect of NTS ablation on baroreceptor reflex activity. Each number represents the mean and standard error obtained from 6 cats. Δ B.P.= change in mean blood pressure (- denotes a decrease and + denotes an increase in BP). Δ H.R. = change in heart rate. B.L.C.O. = bilateral carotid occlusion. N.E. = Norepinepharine PMN= Paramedian Reticular Nucleus. * denotes significance at $P<0.05$ as measured by paired t test. Lesioning stimulus parameters were 2-3 ma/10 sec.

	CONTROL		UNILAT.NTS ABL.		BILAT. NTS ABL.
MEAN B.P.	122.0 ± 7.39		135.0 ± 9.68		124.0 ± 17.83
PEN ↑	Δ B.P.	Δ H.R.	Δ B.P.	Δ H.R.	Δ B.P.
	10V. -52.67 ± 9.58	-11.83 ± 4.50	-58.83 ± 13.17	-11.83 ± 5.54	-73.50 ± 15.60
	5V. -38.67 ± 8.62	-10.33 ± 3.97	-51.67 ± 14.14	-8.33 ± 5.64	-66,00 ± 16.37
B.L.C.O.	+ 52.50 ± 6.91		+ 38.83* ± 7.46		+ 23.67* ± 3.27
NOR EPI	+ 63.83 ± 5.34		+ 59.33 ± 3.86		+ 69.83 ± 9.63

Figure 8

The effect of NTS ablation on the pressor response and heart rate evoked by bilateral occlusion of the carotid arteries in vagotomized cats. Panel A represents the control pressor and heart rate responses to BICO. Panel B represents the cardiovascular responses to BICO in the same cat after unilateral NTS ablation. Panel C shows the responses after bilateral NTS ablation. The downward deflections of the time base represent clamping and unclamping of the carotids which were occluded for about 30 seconds.

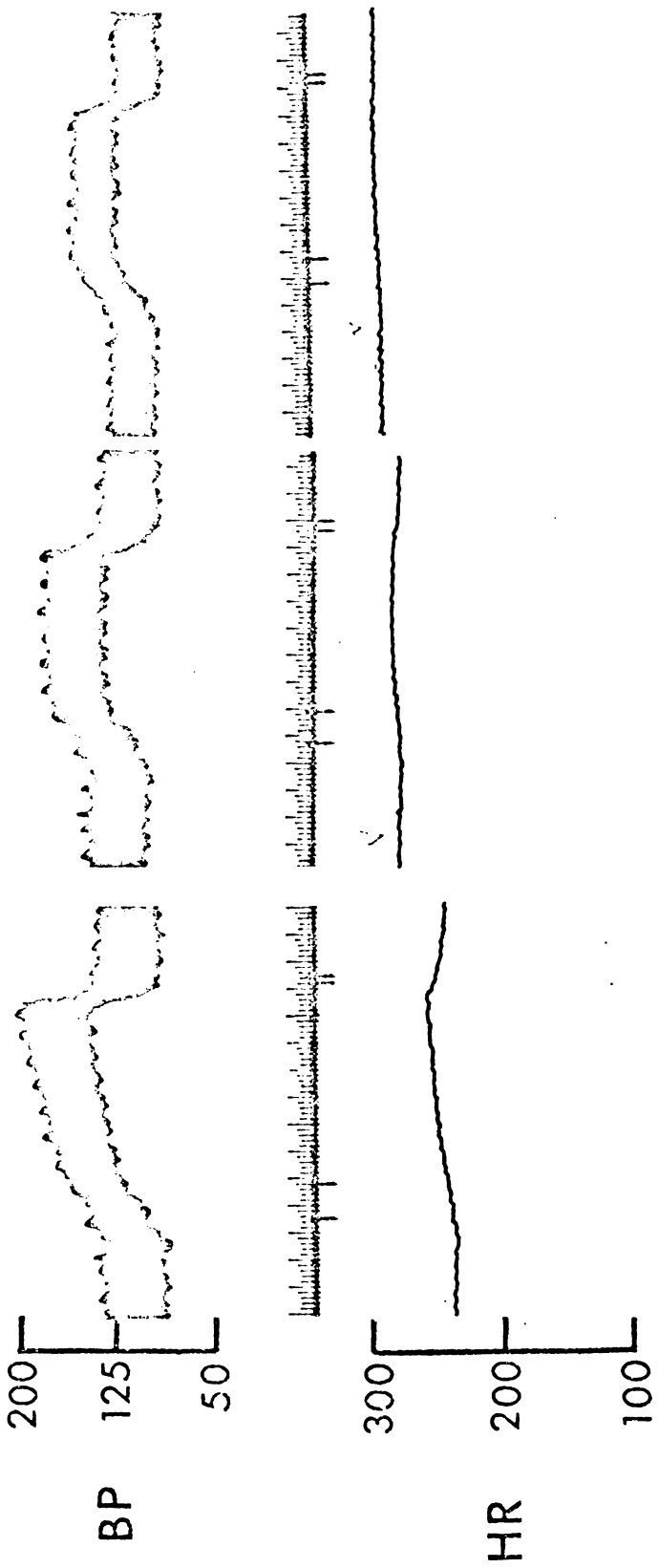

A
B
C
200
125
50
BP
300
200
100
HR

Table 3

The effect of NTS ablation on baroreceptor reflex activity. Each number represents the mean and the standard error obtained in 6 cats. ΔB.P.= the change in blood pressure evoked by NTS stimulation or PIGO. ΔH.R.= the change in heart rate. The values depicted under Post lesion represent the cardiovascular responses 15 minutes after bilateral NTS ablation extending from 0.75 mm below the obex to 0.75 mm above the obex. The values under the heading extended lesions represent the responses 15 minutes after bilateral lesions which extended in 1 mm steps in a rostral direction to a level 2.5 mm above the obex. The lateral coordinant was 0.75 mm from the midline; the dorsal-ventral coordinant was 2.5 mm from the dorsal medullary surface. The lesioning current was 3 ma/15 sec.

	CONTROL	POST LESION (0.175mm ↑ +↓ OBEX)		POST LESION (EXTENDED LESION)	
MEAN B.P.	136.0 ± 10.60	132.0 ± 12.86		125.0 ± 12.41	
NTS	Δ B.P.	Δ H.R.	Δ B.P.	Δ H.R.	Δ B.P.	Δ H.R.
10V.	+ 6.33 ± 12.15	+0.50 ± 5.11	-2.83 ± 8.02	-2.33 ± 3.62	+14.60 ± 13.29	+3.20 ± 2.85
5V.	-7.17 ± 10.94	-4.17 ± 3.60	+0.50 ± 6.53	-0.33 ± 1.67	+9.00 ± 8.34	+0.40 ± 0.40
B.L.C.O.	+60.00 ± 6.85		+51.33 ± 5.08		+53.80 ± 7.20	

Table 4

The effect of dorsal lateral reticular formation ablation on baroreceptor reflex activity. The values under the expt.# represent individual responses. The mean and standard error are given at the right for each group. Post ablation data represents the cardiovascular responses obtained 30 minutes after ablations which were made bilaterally in the DLRF including an area from the obex to 6 mm above the obex; 3 mm lateral of the midline; and 2.5 mm from the dorsal surface. The lesioning parameters were 5 to 10 ma/20 sec.

	CONTROL					POST ABLATION				
EXPT.#	1.	2.	3.	$\bar{X}$	S.E.	1.	2.	3.	$\bar{X}$	S.E.
MEAN B.P.	100.0	125.0	135.0	120.0	±10.41	35.0	75.0	53.0	54.3	±11.57
H.R.	175.0	194.0	220.0	196.3	±13.04	145.0	210.0	124.0	159.7	±25.89
B.L.C.O. ΔB.P.	42	73	52	55.67	± 9.13	5	18	0	7.67	± 5.36
NOR EPI	70	60	105	78.3	±13.64	38	55	100	64.33	±18.49

Table 5

The effect of midline "depressor area" ablation on baroreceptor reflex activity. The individual responses are shown for each of 10 experiments. The mean and standard error are represented at the bottom of each group. An explanation of the lesioned sites is presented in Methods. The heading Lesion 0.5 & 1.5 represents responses obtained 15 minutes following lesioning from the obex to 1.5 mm above the obex; values under Lesion 2.5 & 3.5 represent responses following lesions which attempted to destroy the midline depressor area from the obex to 3.5 mm above the obex; values under Lesion 4.5 represent the responses following lesions from the obex to 4.5 mm above the obex. In some cases following the extended lesions, blood pressure dropped too low to record responses. # = experimental animal number; MBP = mean blood pressure; HR = heart rate; BLCO = bilateral carotid occlusion; and NE = Norepimepharine.

CONTROL

#	MBP	HR	BLCO ΔBP	Δ HR	NE(ΔBP)
1	137	216	53	16	50
2	115	187	25	8	85
3	97	188	78	16	57
4	106	187	35	15	95
5	95	225	76	11	95
6	140	184	66	20	77
7	120	182	39	8	70
8	117	195	72	6	67
9	100	192	75	11	60
10	87	220	31	7	52
X	111.4	197.9	55.0	11.8	70.8
S.E	5.60	5.07	6.60	1.49	5.29

LESION 0.5 & 1.5

MBP	HR	BLCO ΔBP	Δ HR	NE(ΔBP)
167	255	62	16	45
90	187	22	9	95
105	176	46	12	80
107	212	46	10	105
85	225	70	16	97
108	190	50	9	87
150	204	54	8	63
112	193	72	11	72
92	196	71	6	59
113	236	25	5	62
112.9	207.4	51.8	10.2	76.5
8.28	7.79	5.67	1.17	6.15

LESION 2.5 & 3.5

	MBP	HR	BLCO ΔBP	Δ HR	NE(ΔBP)
1	95	272	12	0	55
2	103	220	31	7	75
3	55	144	9	2	118
4	97	222	31	5	142
5	95	225	63	14	117
6	103	188	25	4	140
7	170	212	58	8	61
8	135	202	51	4	67
9	92	202	52	6	82
10	105	246	20	6	70
X	105.0	213.3	35.2	5.6	92.7
S.E.	9.47	10.79	6.16	1.19	10.50

LESION 4.5

MBP	HR	BLCO ΔBP	Δ HR	NE(ΔBP)
95	235	25	4	72
125	225	60	11	120
152	230	40	7	67
127	208	49	3	90
100	215	45	8	55
119.8	222.3	43.80	6.60	84.25
10.30	6.22	5.74	1.43	13.89

Figure 9

Correlation graph relating the change of mean blood pressure levels and the change in the pressor response to BLCO following midline ablation. The ordinant represents the change in mean blood pressure (ΔMBP) following midline ablation extending from the obex to 3.5 mm above the obex. Increases in MBP are represented in the top half of the ordinant; decreases in MBP are shown on the bottom half. The abcissa represents changes in the pressor response (Δ BP) to BLCO following the lesion (positive changes to the right-decreases in pressor responses to the left). Each point represents the difference between control values and values obtained following lesion (n=10). The coefficient of correlation was 0.722 which was significant at the 5% level.

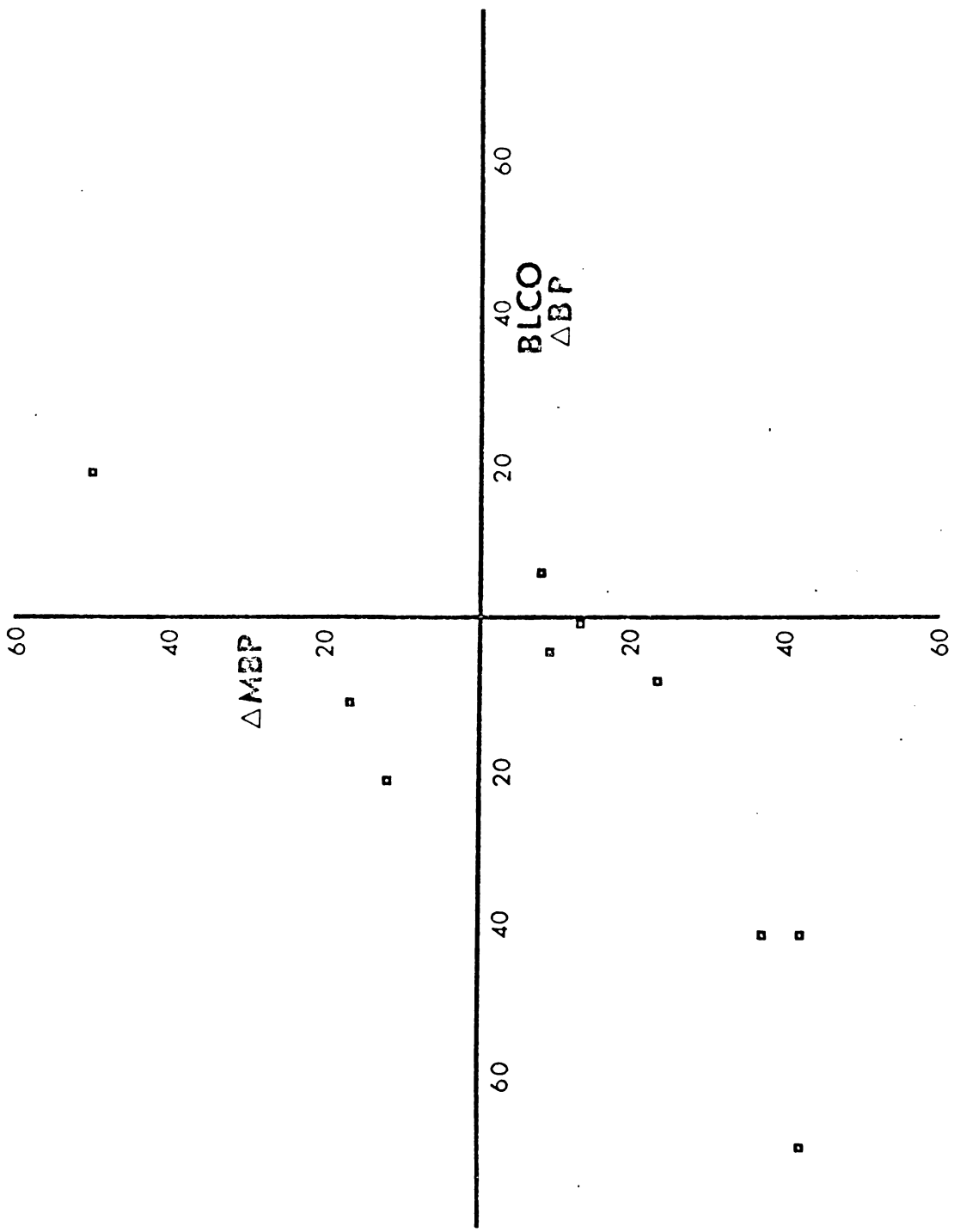
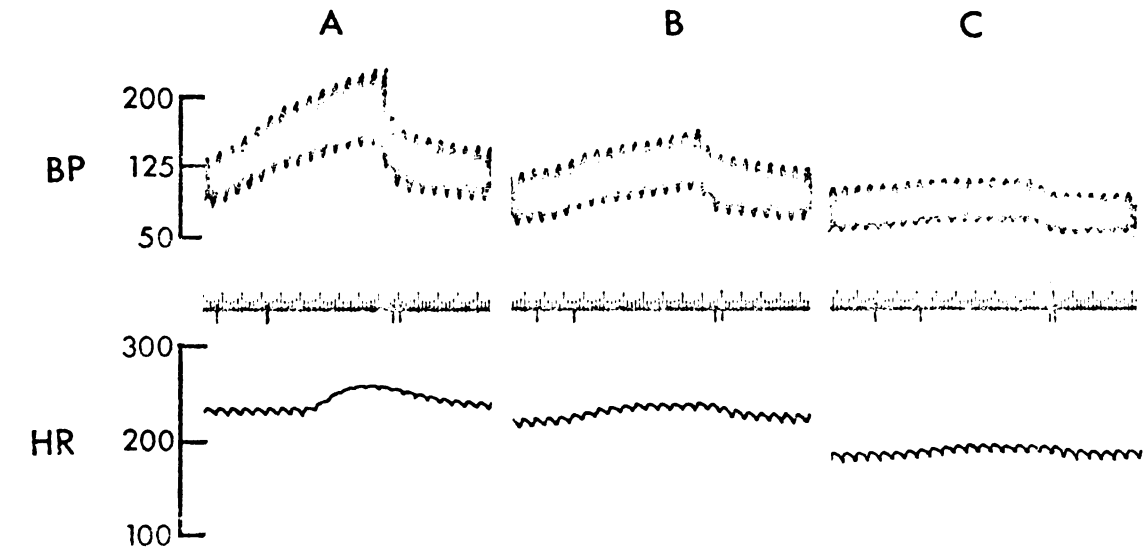

60
40
20
ΔMBP
20
40
60
BLCO
ΔBP
20
40
60

Figure 10

The effect of midline "depressor area" ablation on baroreceptor reflex activity. The top two panels represent the reduction of the pressor response to BLCO following ablation along with heart rate responses. The bottom two panels show the potentiation of the pressor response to NE following ablations along with the heart rate changes. Panel A in both cases represents the control values; Panel B shows the responses observed after midline ablation of 2 mm of the midline from the obex to 2 mm above the obex; Panel C shows the responses following extended midline ablation in the same animal to include an area from the obex to about 4 mm above the obex. The downward deflections from the time base in the top panels represent the clamping and unclamping of the carotids on both sides. The downward deflection in the bottom panels represents the i.v. injection of NE (1 ug/kg).


A
B
C
200
125
50
BP
300
200
100
HR

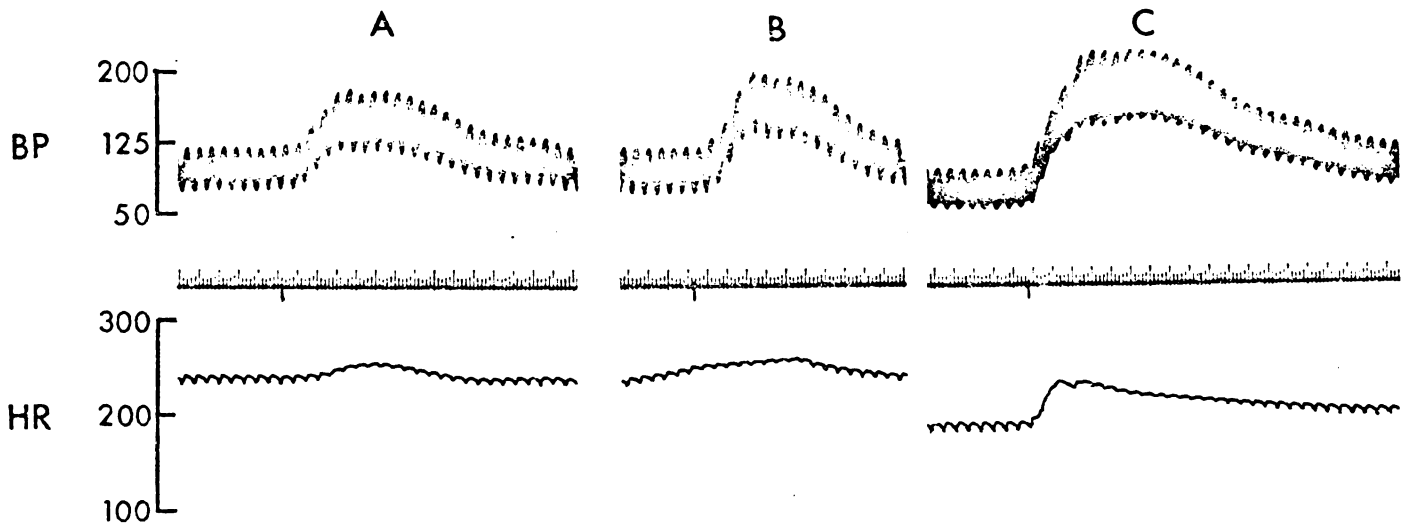

A
B
C
200
125
50
BP
300
200
100
HR

Figure 11

The effect of blood pressure level on the pressor response to BLCO following midline ablation. Panel A represents the control pressor response to BLCO when blood pressure is quite low. Panel B shows the enhanced pressor response in the same cat after lesion in which blood pressure levels have increased. Between panels A & B, midline ablation was performed extending 3.5 mm in the A-P plane. The heart rate changes are also shown. The downward deflections of the time base represent clamping and unclamping of the carotids (about 30 sec. occlusion).

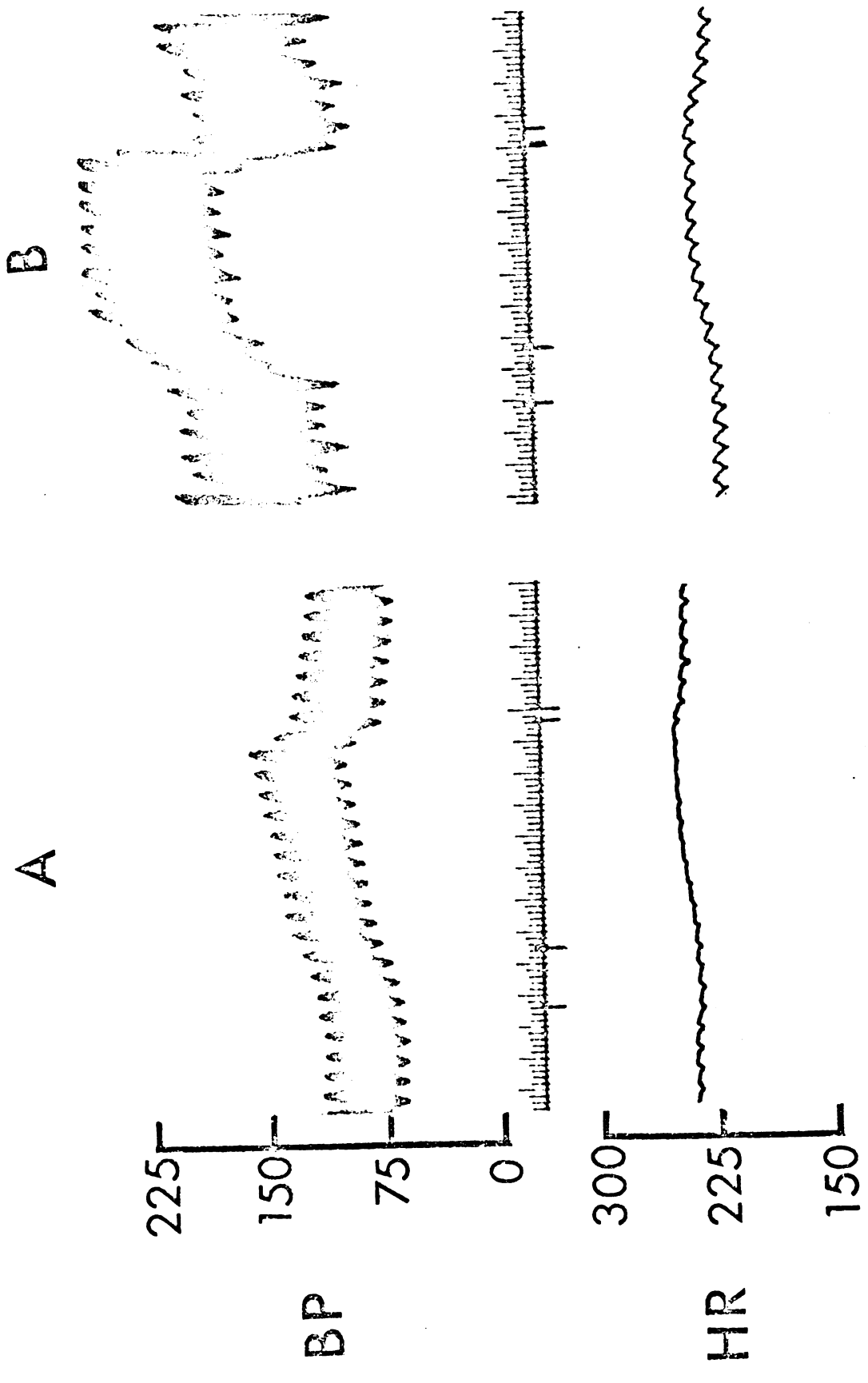

A
B
BP
225
150
75
0
HR
300
225
150

Figure 12

The effect of midline ablation on the cardiovascular responses elicited by NTS stimulation. Blood pressure and heart rate responses to NTS stimulation are represented: Panel A depicts the control cardiovascular responses to NTS stimulation; Panel B shows the responses to NTS stimulation after bilateral vagotomy; Panel C shows the responses in the same cat after midline ablation extending from the obex to about 2 mm above the obex; Panel D represents the responses to NTS stimulation after extended midline ablation to include an area from the obex to about 4 mm above the obex in the A-P plane. The downward deflections of the time base represent the duration of NTS stimulation (about 10 sec.).

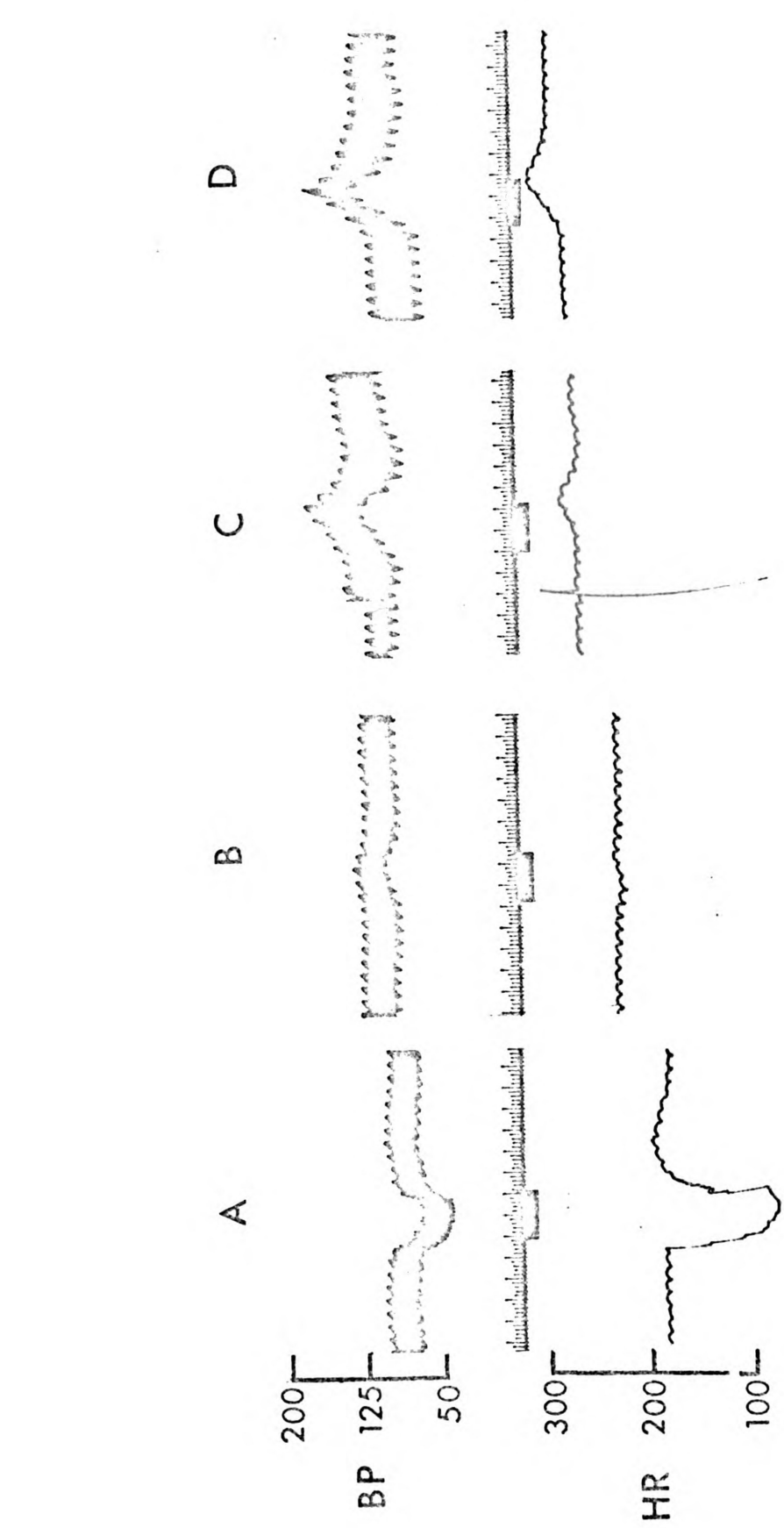

A
B
C
D
200
125
50
BP
300
200
100
HR

Table 6

The effect of midline ablation on cardiovascular responses elicited by NTS stimulation. Each one-third of the table represents a comparison of control NTS responses to the responses seen after midline lesion. 10 V. and 5 V. stimulus intensities were delivered to NTS. The left one-third of the table compares the control responses to responses after midline ablation extending 2 mm (Lesion 0.5 & 1.5 mm). N=10. The middle one-third of the table shows responses from NTS stimulation before and after midline lesion extending about 4 mm (Lesion 2.5 & 3.5mm). N=8. The values on the right side of the table compare control NTS responses to responses seen after midline ablation extending about 5 mm in the A-P plane (Lesion 4.5 mm). N=5. Each value represents the mean and standard error. * denotes significance at the 5% level.

	LESION 0.5 & 1.5 mm		LESION 2.5 & 3.5 mm		LESION 4.5 mm	
	Control	Post Lesion	Control	Post Lesion	Control	Post Lesion
10 V.						
B.P.	8.10±8.89	16.9±6.92	3.63±10.49	23.75±7.77	-11.0±7.94	19.75±5.19
H.R.	1.10±3.51	4.4±3.90	0.88±4.44	6.75±3.86	-6.25±5.54	5.0 ± 4.04
5 V.						
B.P.	-0.67±5.21	6.0±3.12	-1.57±6.21	8.71±1.77	-11.5±3.93	*6.25±1.25
H.R.	-2.89±3.19	1.33±2.10	-2.86±4.04	2.43±1.21	-8.0±4.90	*2.25±2.17

DISCUSSION

The results of the stimulation experiments characterize the cardiovascular responses elicited from proposed medullary sites in the baroreceptor reflex pathway as well as indicate the relative contributions of sympathetic and parasympathetic components. The NTS stimulation data reveals that the middle third of this nucleus mediates both depressor and bradycardia responses. Since vagotomy substantially reduced the bradycardia but not the depressor response, it appears that there is a sympatho-inhibitory component mediating blood pressure changes as well as a parasympathetic vagal component mediating heart rate responses. These findings therefore suggest that NTS is a likely site in the baroreceptor reflex pathway.

Characterization of the cardiovascular responses from PMN stimulation revealed a large depressor response which persisted following vagotomy implying a pure sympatho-inhibitory response. Since the small bradycardia elicited by PMN stimulation was present after vagotomy, it is likely that the vagal component of the baroreceptor reflex does not extend to this nucleus. The fact that large depressor responses were elicited at this site suggests that PMN may be involved in the sympatho-inhibitory pathway of the reflex. An unexpected response occurred, however, when PMN was stimulated at low voltage intensities as shown in figure 6. The large depressor responses evoked at high stimulus parameters were converted to small pressor responses at low intensities. Possible explanations are as follows: 1) There may be pressor neurons located in the classic "depressor area" with low

thresholds which are masked at high stimulus intensities but manifest at low voltages which are subthreshold to surrounding depressor neurons or 2) The large depressor responses may be due to current spread to inhibitory afferents coming in toward PMN and other midline nuclei. At low voltages current spread would be minimal and pressor neurons endogenous to PMN would predominate. This idea that pressor neurons exist near the midline would be compatable with Brodal's anatomical arrangement of the reticular formation which I mentioned earlier. That is, the site of sympatho-inhibition should be located near the midline since only midline reticular formation neurons send axons in a rostral-caudal plane. A descending pathway with long axons to the intermediolateral cell column in the spinal cord is, of course, the effector component of the reflex that regulates vascular tone.

The results obtained from electrical activation of the inferior olivary nucleus indicate that this site may be involved in the sympatho-inhibitory component of the baroreceptor reflex. The fact that depressor responses were observed before and after vagotomy and the observation that heart rate was unaltered by inferior olive stimulation suggest that the cardiovascular responses elicited from this site were uncontaminated by vagal influences.

Since the stimulation experiments revealed cardiovascular responses similar to those characteristic of the baroreceptor reflex, it was decided to ablate these medullary sites and ascertain the effect of ablation on baroreceptor reflex activity induced by bilateral carotid occlusion, i.v. injection of NE, and stimulation techniques as described in Methods, The data presented in Table 2 reveal that NTS is an essential

nuclear substrate in the baroreceptor reflex pathway as ablation of this site prevents the compensatory reflex adjustments to baroreceptor activation. This is shown by the significant reduction of the BLCO response following ablation. When the carotids are clamped, tonic sympatho-inhibitory impulse traffic is essentially abolished with a concomitant large pressor response. If the baroreceptor reflex pathway is interrupted one would see less of a release of inhibition and, consequently, a reduction of the pressor response. Of course, the possibility exists that the ablation may be interrupting the CSN afferents in their course toward NTS in the tractus solatarius. In this case a maximum reduction of the BLCO response would be expected. Another finding suggesting NTS involvement in the pathway is that the depressor response to NTN stimulation is substantially potentiated after ablation. This potentiation indicates that NTS ablation prevents reflex adjustments to the decrease in blood pressure and, consequently, enhancement of the response is seen. Another indication of NTS involvement in the baroreceptor reflex pathway is that the pressor response to i.v. injection of NE is slightly augmented following bilateral NTS ablation. Further evidence implicating NTS in the pathway is revealed by the mean blood pressure levels: Mean blood pressure is increased after ablation, however not significantly. One might expect a greater increase in blood pressure levels after NTS ablation if this site was involved in the baroreceptor pathway since sites mediating tonic inhibitory input to pressor regions would be eliminated. Three factors may be masking the increase: 1) trauma induced during the lesioning process, 2) damage to pressor neurons surrounding NTS which would tend to lower pressure, and 3) persistance of a portion of the baroreceptor

reflex fibers (as indicated by retention of 45% of the response) which would tend to compensate for a rising pressure.

Since the ablation experiments were performed on vagotomized cats it was impossible to assess the parasympathetic contribution to the baroreceptor reflex. However, since the stimulation data on NTS revealed a significant vagal component, it would be expected that heart rate would rise following ablation of NTS.

The data obtained from PMN ablation experiments indicates that the lesioned area does not contribute significantly to the sympatho-inhibitory pathway of the baroreceptor reflex. The pressor response to BLCO was reduced only 10% following bilateral ablation of the area of PMN receiving monosynaptic afferent CSN projections as described by Miura and Reis. Also, the observation that PMN ablation did not significantly alter blood pressure responses to NTS stimulation implies that the ablated site did not interfere with baroreceptor reflex function. In addition, one would expect mean blood pressure to increase after ablation if PMN were involved in the sympatho-inhibitory pathway of the reflex. The fact that blood pressure decreased following the lesion may indicate that some pressor neurons are located in midline areas and were damaged in the ablations. This supports earlier evidence which was cited showing a pressor response from PMN stimulation at low parameters. It is difficult to reconcile these results with the electrophysiological data of Miura and Reis and the earlier experiments I described showing a pronounced depressor response following PMN stimulation. However, the possibility exists that the CSN afferent fibers traced to PMN are not serving a sympatho-inhibitory function,

but rather are chemoreceptor afferents or afferent fibers modulating EEG or spinal somatic reflexes.

Midline ablations were carried out to assess the role of this area in the baroreceptor reflex. Several reports based on electrophysiological evidence have shown that baroreceptor afferents or their secondary projections terminate in midline areas: (Humphrey, 1967, Crill and Reis, 1968, Sampson and Biscoe, 1968, and Miura and Reis, 1969). Several lines of evidence obtained in the present experiments indicate that the midline area is a crucial substrate for the sympatho-inhibitory component of the baroreceptor reflex: 1) The pressor response to BLCO was significantly reduced following midline ablation. In one cat, however, it was noted that the BLCO response was potentiated after ablation in which blood pressure had increased from control level. In this case, one might conclude that the midline ablation did not interfere with the baroreceptor reflex compensatory mechanisms, however, an explanation may be that as pressure is increased there is more input along the baroreceptor afferents and, therefore, occlusion of the carotids would suddenly reduce this input with a concomitant large pressor response. That is, at high blood pressure levels there would be a greater release of tonic sympathetic activity upon occlusion of the carotids since the sympatho-inhibitory input had been essentially eliminated. 2) The pressor response to i.v. injection of NE was significantly enhanced following midline ablation. This implies that the baroreceptor reflex pathway is interrupted so as not to be able to compensate for the rise in blood pressure produced by NE. 3) The depressor responses or small pressor responses elicited by stimulation

of NTS were converted to larger pressor responses after midline ablation. Since NTS lies well within the "pressor" areas of the medullary reticular formation the blood pressure response from this site is probably a combination of two elements: Stimulation of neurons in the baroreceptor pathway which would result in a decrease in blood pressure, and stimulation of pressor neurons surrounding NTS which, of course, would produce a rise in blood pressure. The observation that small pressor responses are potentiated following ablations might indicate that the ablation is interrupting baroreceptor pathways or terminations in the midline so as to prevent the sympatho-inhibitory functions. This would allow the pressor neurons surrounding NTS to dominate the response unimpeded by compensatory baroreceptor function.

Finally, it was noted in most of the animals that resting blood pressure levels were decreased after progressive midline ablations. This may provide further evidence that tonic pressor neurons are located in midline depressor areas, and their subsequent destruction after ablation may account for the reduction in baseline blood pressure levels.

The concept of pressor neurons in the midline "depressor regions" is a possibility that is compatable with several lines of research. Kahn and Mills (1967) evoked small pressor responses in midline medullary areas associated with an inhibition of splanchnic nerve activity. They showed that this pressor response was potentiated (additional 30 mm Hg rise in BP) during prolonged carotid occlusion implying that the area stimulated interacted with baroreceptor input. This study

indicates that midline medullary stimulation selectively activates and inhibits different parts of the sympathetic vasomoter outflow.

As described in the introduction, Salmoiraghi (1962) located several "type II" cardiovascular neurons in the midline medullary areas. These neurons decreased their discharge rate as blood pressure increased. That is, they appeared to be inhibited by pressoreceptor discharge. It appears possible that these type II neurons may be pressor neurons in the midline and represent a site of sympatho-inhibition of the vasomoter outflow to the vessels.

Sampson and Biscoe (1968) detected several neurons in the midline area which exhibited intracellular IPSP's during CSN stimulation. These could be tonic pressor neurons which are inhibited by baroreceptor input. Of course, the possibility exists that these are inhibitory interneurons in the baroreceptor pathway that inhibit tonic pressor outflow at another site.

Evidence from the present experiments includes: 1) Stimulation of midline medullary areas (MN) elicited pressor responses at low voltage intensities. 2) Blood pressure decreased following MN ablation possibly indicating that pressor neurons were interrupted in the ablation proceedure. 3) Blood pressure fell following midline "depressor area" ablations.

Assuming pressor neurons may exist in midline "depressor areas", one must reconcile the existance of two pressor pools of neurons in the medulla. The possibility exists that there are two brainstem mechanisms involving pressor neurons which mediate cardiovascular function: A midline pool which discharges tonically to baroreceptor

afferent input as well as phasically to supramedullary afferents that modulate the reflex; and a pressor pool in the dorsal lateral reticular formation which contributes to the normal vascular tone through intrinsic mechanisms. Both pressor outputs would converge on the final common neuronal locus in the intermediolateral horn of the spinal cord with the midline tract having the greater influence on the discharge of these cord neurons. This idea would be compatable with the following research findings: 1) Humphrey was unable to detect potentials in "pressor" areas of the dorsal lateral reticular formation following CSN stimulation implying that baroreceptor afferents modify tonic vasomotor activity at a site outside of the classic pressor areas of the medulla. 2) The supramedullary modifications of the sympatho-inhibitory component of the baroreceptor reflex appear to occur in midline areas in the medulla rather than in the lateral pressor areas. 3) Chai and Wang (1968) were never able to show an increase in blood pressure following extensive lesions in the dorsal lateral reticular formation which they claimed interrupted baroreceptor reflex pathways. 4) Manning (1965) has shown that extensive lesions in the medullary "vasomotor area" (DLRF) failed to alter the cardiovascular reflex adjustments to baroreceptor reflex activation.

Finally it should be mentioned that a spinal site of sympatho-inhibition has been suggested by several research groups. Experiments have demonstrated that animals following recovery from cervical cord transection regain nearly normal blood pressure levels and become capable of certain cardiovascular reflex responses. Thus, it appears that spinal cardiovascular neurons in the intermediolateral columns

can take over for medullary vasomotor centers. These spinal centers may be functioning in the intact animal as the primary tonic and reflex regulators of vascular function with the medullary cardiovascular neurons serving only to modulate this activity. On the other hand, spinal regulation of cardiovascular function may occur only when higher centers are functionally depressed as in the spinal animal and thus, not exert any control in the normal physiological state.

In summary, it appears 1) that NTS based on stimulation and ablation experiments is an important relay site in the baroreceptor reflex pathway, mediating both sympatho-inhibition and vagal activation.
2) Paramedian reticular nucleus is probably not an important relay nucleus in the baroreceptor reflex pathway mediating sympatho-inhibitory functions. The baroreceptor afferents traced to this site by Miura and Reis may be fibers modulating EEG activity or influencing spinal somatic reflexes.
3) It was shown that the vagal component of the baroreceptor reflex mediating heart rate changes does not extend to midline areas.
4) It appears that classic "midline depressor areas" participate in the baroreceptor reflex pathways mediating sympatho-inhibition. Responses to baroreceptor activation following midline ablation indicated that the lesioned sites were involved in the compensatory reflex adjustments mediated by the barorceceptor reflex pathways.
5) Evidence was presented that pressor neurons may reside in classic "depressor regions" of the medulla and may constitute the site of baroreceptor induced sympatho-inhibition to the blood vessels.

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