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The Role Of The Hypothalamus In The Mediation And Central Control Of Cardiovascular Reflexes In The Cat

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THE ROLE OF THE HYPOTHALAMUS IN THE MEDIATION AND CENTRAL CONTROL OF CARDIOVASCULAR REFLEXES IN THE CAT

by

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ABSTRACT

This study was done to identify the neural structures involved in hypothalamic inhibition of reflex vagal bradycardia.

A discrete vasomotor region in the hypothalamus was identified in chloralose anaesthetized cats by studying the effect of hypothalamic stimulation on heart rate and arterial pressure, by recording the response of single hypothalamic neurones to selective stimulation of baroreceptors and chemoreceptors, and by demonstrating the inhibitory effect of stimulation of this region on reflex vagal bradycardia. Electrical stimulation of a discrete region in the posteromedial hypothalamus (PMH) in nine cats was found to elicit arterial hypertension and cardioacceleration that was abolished by administration of propranolol (1.5 mg/kg i.v.). In 14 cats, the spontaneous activity of 23 of 51 single units recorded in the PMH was shown to be altered by electrical stimulation of the carotid sinus nerve (CSN): 16 of these units were inhibited and seven were excited. Stimulation of baroreceptors (noradrenaline, 0.5 - 2.0 µg/kg i.v.) decreased the spontaneous activity of units inhibited by CSN stimulation. Excitation of right carotid body chemoreceptors (intracarotid injection of sodium cyanide, 50-100 µg/kg) increased the discharge frequency of units excited by CSN stimulation. In 18 cats, transected at C_7, electrical stimulation of the right and left PMH was found to consistently inhibit reflex vagal bradycardia elicited by electrical stimulation of the right CSN and by selective stimulation

of baroreceptors and right carotid body chemoreceptors. In addition, it was shown that the PMH probably exerts a tonic inhibitory effect on reflex vagal bradycardia, as the cardiac slowing elicited by chemoreceptor stimulation was increased in magnitude by midcollicular decerebration in four cats.

To identify medullary structures involved in hypothalamic inhibition of reflex vagal bradycardia the medulla was systematically explored for vagal cardioinhibitory sites and the effect of simultaneous stimulation of the PMH on the magnitude of this bradycardia was investigated. Electrical stimulation of the right medulla in 27 chloralosed spinal cats (C_7) elicited vagal bradycardia from 160 histologically confirmed sites. The majority of the sites were located in four nuclei: n. ambiguus (NA, 55 sites); ventral n. medullae oblongatae centralis (MOC, 31 sites); n. of tractus solitarius (NTS, 23 sites); and dorsal MOC (18 sites). Ipsilateral vagotomy abolished the bradycardia elicited by stimulation of the NA (11 cats), the NTS (4 cats) and the ventral MOC (3 cats). In six experiments administration of sodium pentobarbital (10 mg/kg i.v.) reduced the magnitude of the bradycardia elicited from the NTS significantly more than the bradycardia from the NA and from the peripheral vagus. Electrical stimulation of the ipsilateral or contralateral PMH in nine cats was found to inhibit the vagal bradycardia elicited by stimulation of the right NTS and right dorsal MOC but not the bradycardia elicited from the NA.

To investigate the sites of termination of inhibitory hypothalamo-medullary pathways field potentials were recorded in the

medulla during electrical stimulation of the PMH. Stimulation of the right and left PMH in 21 spinal cats elicited field potentials at 405 sites in the ipsilateral and contralateral medulla. The field potentials were located in the inferior olivary n., lateral reticular n., NTS, ventral and dorsal MOC, NA, parahypoglossal area and dorsal longitudinal fasciculus. The field potentials had a peak latency of 19-53 msec, did not follow frequencies of stimulation greater than 26 Hz, were not affected by muscle paralysis, but were abolished by barbiturate (sodium pentobarbital, 20 mg/kg i.v.) and by asphyxia. Ipsilateral hemitransection at the pontomedullary junction abolished ipsilateral field potentials and reduced contralateral field potentials.

It is concluded that the PMH, in addition to the medulla and pons, mediates baroreceptor and chemoreceptor reflexes and that the inhibitory effect of PMH stimulation on reflex vagal bradycardia is mediated by crossed and uncrossed pathways which alter the electrical activity of neurones in discrete nuclear structures located in the medulla..

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INTRODUCTION

It is generally recognized that many portions of the neuraxis exert an influence of the cardiovascular system and that the essential components mediating cardiovascular reflexes are located in the pons and medulla. However, the localization of brain stem structures and the study of mechanisms involved in these reflexes has occurred only because of the introduction of new experimental techniques. The technique of electrical stimulation led to new advances because the physiological function of a structure could be inferred from the response elicited by artificial stimulation of that structure. Using this technique Weber & Weber (1845) demonstrated that stimulation of the peripheral end of the vagus and of the medulla elicited a decrease in heart rate. Cyon & Ludwig (1866) stimulating the aortic depressor nerve demonstrated that cardiovascular changes could also be elicited by altering the afferent input to the central nervous system. To localize the vasoconstrictor and vasodilator centres postulated by Bayliss (1893), several authors, during the first part of this century, observed that cardiovascular changes could be elicited by electrical stimulation of the floor of the fourth ventricle (eg. Miller & Bowman, 1915; Ranson & Billingsley, 1916). In 1939, Wang & Ranson systematically stimulated structures within the brain stem using the stereotaxic technique (Horsley & Clarke, 1908) and located general regions at which electrical stimulation elicited either an increase or a decrease in arterial pressure.

The introduction of a technique for accurately localizing the

site of an electrode tip within the central nervous system (Marshall, 1940) and the development of a method for recording evoked potentials (Adrian, 1926; 1937) facilitated the localization of several medullary nuclei that received afferent fibres from the carotid sinus nerve (Humphrey, 1967). Similarily, Crill & Reis (1968) located several discrete medullary nuclei, electrical stimulation of which elicited antidromic evoked potentials in the carotid sinus and aortic depressor nerves.

The development by Nauta & Gygax (1951) of a technique for tracing fibres anatomically in the central nervous system enabled Smith (1965) to demonstrate a direct descending projection from an area in the posterior hypothalamus, stimulation of which elicited an increase in arterial pressure and cardioacceleration, to several medullary nuclei and to the intermediolateral region of the thoracic spinal cord. In addition, using the same technique Cottle (1964) was able to locate the site of termination of primary afferent cardiovascular neurones in the medulla of cats and Morest (1967) traced pathways from this site to other medullary and pontine nuclei possibily involved in cardiovascular reflex arcs.

In an attempt to explain the various experimental observations, various concepts of central neural control of the cardiovascular system have been proposed.

The classical view of the functional organization of cardiovascular control proposes that the main integrating area is found in a diffuse network of reticular neurones in the pons and medulla (Alexander, 1946; Bach, 1952; Wang & Chai, 1967). On the basis of

experiments in which these regions were electrically stimulated the medulla and pons have been roughly subdivided into a ventromedial "depressor centre" and a dorsolateral "pressor centre" (Alexander, 1946). The diffuse regions are presumed to maintain an adequate level of arterial pressure by controlling the discharge of vasoconstrictor neurones in the spinal cord, and to receive excitatory and inhibitory inputs from peripheral visceral and somatic afferents and from supra-pontine structures. This concept is found in current textbooks of physiology (Mountcastle, 1968; Ruch & Patton, 1965).

Recently, this traditional concept of central control has been re-evaluated. A more flexible regulatory system has been proposed by Peiss (1965) who views central control of the cardiovascular system as being mediated by a group of interacting parallel mechanisms corresponding topographically to areas of the neuraxis which have been found to influence the cardiovascular system. Each of these areas has a separate afferent input and a separate efferent projection via the spinal cord or vagus to the heart and blood vessels. In particular, it has been proposed that the hypothalamus and the lower brain stem can independently maintain an adequate level of arterial pressure and possess an afferent information channel and an effector limb.

With regard to the hypothalamus the amount of information on the role of this structure in the control of the cardiovascular system is extensive and has been reviewed recently (Folkow & Neil, 1971). The accepted view is that the hypothalamus contains a number of "centres" which are responsible for well integrated response patterns, including the appropriate cardiovascular adjustments (Folkow & Neil,

1971). Reports in the literature regarding the effects of stimulation of the hypothalamus on cardiovascular parameters do not provide information on the precise locations of the sites stimulated and on the neural mechanisms involved in the observed responses (Abrahams, Hilton & Zbrozyna, 1960; Eliasson, Folkow, Lindgren & Uvnas, 1951; Kabat, Magoun & Ranson, 1935; Manning & Peiss, 1960; McQueen, Brown & Walker, 1954). However, it may be suggested that some of the effects of hypothalamic stimulation are mediated by a descending hypothalamospinal pathway influencing sympathetic preganglionic neurones directly. This possibility is supported by the demonstration of a diffuse descending projection, which has been traced by the Nauta technique, from the posterior hypothalamus to the region of the lateral column of the spinal cord (Smith, 1965).

In addition, the hypothalamus has been shown to exert an influence on ongoing cardiovascular reflexes (baroreceptor reflex) mediated by lower brain stem mechanisms (Djojosugito, Folkow, Kylstra, Lisander & Tuttle, 1970; Gebber & Snyder, 1970; Hilton, 1963; Hockman, Talesnik & Livingston, 1969; Humphreys, Joels & McAllen, 1971). This latter function of the hypothalamus is well established but it is not known at what level of the baroreceptor arc in the medulla the hypothalamic influence takes place, although it has been reported that hypothalamic stimulation may produce inhibition by depolarization of primary carotid sinus nerve afferent fibres (Weiss & Crill, 1969). Although the possibility exists that the primary afferent depolarization recorded in the carotid sinus nerve may have involved chemoreceptor fibres as well as baroreceptor fibres, it is not known if the hypo-

thalamus can influence the mediation of the chemoreceptor reflex.

With regard to the medulla, it is well documented that reflex cardiovascular changes elicited by stimulation of baroreceptors and chemoreceptors are mediated at this level of the brain stem (Lindgren, 1961; Reis and Cuénod, 1965; Wang and Chai, 1967). However, the structures as well as the precise location of the cardioinhibitory neurones involved in the vagal component of these reflexes is uncertain (Achari, Downman & Weber, 1968; Borison & Domjan, 1970; Calaresu & Pearce, 1965b; Gunn, Sevelius, Puiggari & Myers, 1968).

As much of our knowledge of the neural mechanisms involved in the regulation of the cardiovascular system is based on experimental evidence obtained by ablation or electrical stimulation of relatively large and poorly localized areas in the central nervous system the localization of a discrete region in the hypothalamus, electrical stimulation of which produced reproducible changes in heart rate and arterial pressure was considered a necessary prerequisite for an investigation of the role of the hypothalamus in the central control of cardiovascular reflexes. After localizing a discrete vasomotor region in the hypothalamus experiments were done to investigate the possible role of this hypothalamic region in the mediation of cardiovascular reflexes. Attempts were made to determine if baroreceptor and chemoreceptor inputs projected to this hypothalamic region and to determine if electrical stimulation of this region altered the magnitude of the cardiovascular response to barorecptor and chemoreceptor stimulation. In addition, as the medullary structures mediating cardiovascular reflexes are poorly defined, attempts were made to localize those

medullary sites involved in the mediation of reflex vagal bradycardia. Finally, to investigate the possible medullary sites at which the hypothalamus inhibited the vagal component of the baroreceptor and chemoreceptor reflex attempts were made to study the effect of hypothalamic stimulation on the magnitude of the vagal bradycardia produced by stimulation of localized medullary regions and, in addition, to record evoked potentials in these medullary regions during stimulation of the hypothalamus.

HISTORICAL REVIEW

A. Central neural control of the Cardiovascular System

(a) Medullary control

In the first investigation of the influence of the central nervous system on the heart and blood vessels Philip (1818) observed in the frog that crushing the brain or pithing the spinal cord produced a decrease in heart rate. He also observed cardioacceleration during application of "spirits of wine" to the surface of the brain or spinal cord of the frog and rabbit. In 1845 Weber & Weber observed a decrease in heart rate during electrical stimulation of the vagus in frogs and in rabbits and demonstrated that this response could also be elicited by electrical stimulation of the medulla. In 1888 Laborde stimulated the medulla of the cat by piqûre and demonstrated that the cardioinhibitory area stimulated by Weber & Weber was located in the floor of the fourth ventricle near the nuclei of the IX th, X th, and XII th cranial nerves. In addition to cardioinhibition, the medulla was shown by Dittmar (1873) to be involved in the maintenance of resting arterial pressure. In his studies, Dittmar demonstrated that the greatest fall in arterial pressure produced by transection of the brainstem occurred when the section was performed in the region of the rostral medulla.

To account for the changes in arterial pressure which he observed during stimulation of the medulla, Bayliss (1893) postulated

the existence of reciprocally interacting medullary vasodilator and vasoconstrictor centres and suggested that the increase in diameter of blood vessels observed during medullary stimulation was due to excitation of vasodilator fibres and inhibition of vasoconstrictor fibres.

In 1900 Deganello observed in the dog that application of hot and cold saline to the medulla in the area of the dorsal nucleus of the vagus produced bradycardia and suggested that this nucleus was the cardioinhibitory centre. Miller & Bowman (1915) electrically stimulated the medulla of dogs transected at C_1 and localized the cardioinhibitory centre in the dorsal vagal nucleus. Ranson & Billingsley (1916) described two areas in the floor of the fourth ventricle of the cat which they called the pressor and depressor points. The pressor point, stimulation of which elicited hypertension, was located "at the apex of the fovea inferior"; the depressor point, stimulation of which elicited a decrease in arterial pressure, was situated in the area postrema just lateral to the obex. These authors suggested that the two points corresponded to the vasoconstrictor and vasodilator centres of Bayliss. In 1923, Scott & Roberts demonstrated that the cardio-inhibitory centre of Miller & Bowman was identical to the depressor point of Ranson & Billingsley, as stimulation of the depressor point with the vagi intact produced in addition to the hypotension a marked decrease in heart rate. As cardiac slowing and arterial hypotension were elicited by electrical stimulation of the same point, Scott & Roberts suggested that the depressor point was not the vasodilator centre of Bayliss but rather the location where the afferent fibres of the

depressor reflex arc were near the surface of the medulla. This suggestion was supported by their observation that the same response could be elicited by stimulation of the central cut end of the vagus nerve. To test the hypothesis of Scott & Roberts that cardiovascular responses elicited by stimulation of the surface of the medulla were due to activation of deeper structures Wang & Ranson (1939a), using the Horsley-Clarke stereotaxic instrument, stimulated the brain stem of cats from the pons to the pyramidal decussation and located many points within the bulbar reticular formation at which low intensity stimulation elicited near maximal pressor and depressor responses. Their investigation was followed by a number of similar explorations (Monnier, 1939; Alexander, 1946; Bach, 1952). Bach (1952) made a comparison of the location of pressor and depressor points obtained in his experiments with those of other experimenters and concluded that pressor points were usually located in the dorsomedial and ventrolateral portions of the rostral brain stem, and lateral portions of the caudal brain stem and that depressor points were found most often in the ventromedial portions of the brain stem. Alexander (1946) however, in a comprehensive investigation of the role of the medulla in cardiovascular function placed pressor points in the rostral medulla and depressor points almost entirely within the medial portion of the caudal medulla. Alexander's restriction of pressor points to the rostral medulla and depressor points to the caudal medulla was based on his observation of the changes in resting arterial pressure elicited by serial transection of the brain stem. He found that transection of the brain stem at the pontomedullary junction did not alter resting

arterial pressure. However transection at the level of the obex decreased resting arterial pressure and subsequent transection of the spinal cord at C_1 resulted in an increase in arterial pressure. On the basis of these findings, Alexander concluded that spinal sympathetic vasoconstrictor neurones received a tonic excitatory input from structures located in the rostral medulla and a tonic inhibitory input from structures in the caudal medulla.

In an attempt to locate the site of origin of cardioinhibitory neurones, Harrison & Bruesch (1945) recorded evoked potentials in the region of the obex of cats during electrical stimulation of the cervical vagus. Similar results were obtained by Anderson & Berry (1956) in the same species, and by Lam & Tyler (1952) in the rabbit. However, no precise localization of the site of origin of cardioinhibitory neurones was obtained in these studies because of lack of accurate methods for marking the sites of recording. Urabe & Tsubokawa (1960), by marking sites of recording with small electrolytic lesions at the tip of the electrode, localized evoked potentials elicited by stimulation of the cervical vagus in the nucleus of the tractus solitarius, the nucleus ambiguus and several reticular nuclei but failed to elicit any activity in the dorsal nucleus of the vagus. Porter (1963), also using cats, obtained similar results. Calaresu & Pearce (1965a) recorded unit activity in the dorsal nucleus of the vagus and found seven units which increased their frequency of discharge during reflex bradycardia; however, when the dorsal nucleus of the vagus in the cat was stimulated no changes in heart rate were observed (Calaresu & Pearce, 1965b). Gunn, Sevelius, Puiggari & Myers (1968) observed that stimulation of

the dorsal nucleus of the vagus occasionally resulted in vagal bradycardia in the dog but never in the cat; they observed, however, that stimulation in or near the nucleus ambiguus consistently elicited evoked potentials in the cervical vagus and resulted in cardiac slowing in both the cat and the dog. In view of these results, the earlier contention that the site of origin of cardioinhibitory neurones is located in the dorsal nucleus of the vagus must be reconsidered.

Recently, other medullary structures have been shown to possess a cardioinhibitory function: Achari, Downman, & Weber (1968) electrically stimulated selective areas in the cat brain stem and traced a pathway from which bradycardia could be elicited extending through the reticular formation from the nucleus reticularis pontis caudalis to the nucleus reticularis gigantocellularis.

In summary, by the end of the 19th century, it had been demonstrated by electrical stimulation and piqûre that the cardioinhibitory centre was located in the caudal region of the medulla. In addition, by serial transection experiments Dittmar had demonstrated the tonic activity exerted by the medulla on the heart and blood vessels. Alexander located the origin of this tonic activity. He found that structures in the rostral medulla elicited a tonic excitatory effect on spinal sympathetic neurones and that a tonic inhibitory effect originated in structures located in the caudal medulla. Recently, the development of accurate methods for determining the site of stimulation and recording has facilitated the study of discrete medullary structures involved in cardiovascular control. The recording and stimulation experiments of Urabe & Tsubokawa, Porter,

Calaresu & Pearce and Gunn et al have demonstrated that the nucleus and tractus solitarius and possibly the nucleus ambiguus are involved with cardiovascular control. Their experiments have also caused reconsideration of the earlier contention that the cardioinhibitory centre was located in the dorsal nucleus of the vagus. Finally, the demonstration by Achari et al of a cardioinhibitory pathway in the medulla has pointed to the nucleus reticularis gigantocellularis and nucleus reticularis pontis caudalis as possible sites involved in cardiovascular control.

(b) Hypothalamic control

One of the first indications that supramedullary structures exert an influence on the cardiovascular system was the extensive work done by Karplus & Kreidl between 1909 and 1927 who showed that electrical stimulation of the hypothalamus in the cat elicited marked increases in heart rate and arterial pressure. Although their results were confirmed by Beattie, Brow & Long (1930a, b) and by Allen (1931) in the rabbit it was not until 1935 that a systematic exploration of the hypothalamus was made. In 1935, Kabat, Magoun & Ranson, using the Horsley-Clarke technique, observed that an increase or decrease in arterial pressure could be elicited by stimulation of many sites in the hypothalamus of the cat. Similar results were obtained by Hess (1957), in the awake cat, using the Prussian blue reaction to localize the sites of stimulation.

With regard to the hypertensive response the sites eliciting an increase in arterial pressure were located in the region of the fornix

and fields of Forel. These results were confirmed in the dog by McQueen, Brown & Walker (1954) who also elicited increases in arterial pressure by electrical stimulation of the dorsal and posterior hypothalamus. In 1960, Manning & Peiss observed that in addition to arterial hypertension an increase in heart rate was elicited during stimulation of the posterolateral hypothalamus in the cat. Similar results were obtained by Fuster & Weinberg (1960) and by Folkow & Rubenstein (1966). These cardiovascular responses have been shown to be mediated by the sympathetic nervous system as an increase in neural activity has been recorded in the sympathetic nerves to the heart, spleen, kidney and skeletal muscles of the cat during hypothalamic stimulation (Bronk, Pitts & Larrabee, 1940; Ninomiya, Judy & Wilson, 1970; Pitts, Larrabee & Bronk, 1941).

With regard to the hypotensive response, Kabat et al (1935) demonstrated that stimulation of a region in the preoptic area produced a decrease in arterial pressure. McQueen et al (1954) expanded the responsive region to include the lateral hypothalamus and medial preoptic tract. In 1959, Folkow, Johansson & Oberg elicited depressor responses during stimulation of the anterior hypothalamus. Although it had been shown by Beattie (1932) that the anterior hypothalamus was involved in the control of parasympathetic induced changes in stomach and bladder motility, Folkow et al (1959) suggested that the hypotensive response elicited by stimulation of this region was mediated by the sympathetic nervous system as the response could be elicited in vagotomized cats. This suggestion is supported by the recent demonstration that stimulation of the anterior hypo-

thalamus inhibits efferent sympathetic activity (Hilton & Spyer, 1968; 1971).

In view of the similarity of the cardiovascular responses elicited by hypothalamic stimulation to the cardiovascular changes observed during the emotional states of fear and anger (Adams, Baccelli, Mancia & Zanchetti, 1969; 1971) and during physical activity (Rushmer, Franklin, van Citters & Smith, 1961; Rushmer, Smith & Franklin, 1959) the hypothalamus has been suggested as a site of integration of these cardiovascular changes. Support for this suggestion is the demonstration that the hypothalamus is required for the mediation of tachycardia and arterial hypertension observed during sham rage (Bard, 1930) and that lesions in the hypothalamus decrease the cardiovascular response to exercise in the dog (Smith, Jabber, Rushmer & Lasher, 1960).

In summary, two regions in the hypothalamus have been shown to exert an influence on the cardiovascular system. The anterior hypothalamus is involved in parasympathetic excitation and sympathetic inhibition while the posterolateral hypothalamus is involved in sympathetic excitation. However, the extent of these hypothalamic regions are unknown due to the failure to accurately localize the sites of stimulation.

(c) Hypothalamo-medullary connections

The first investigation of descending pathways from the hypothalamus was done by Beattie, Brow & Lang (1930a, b) who placed lesions in the posterior hypothalamus of the cat and traced degenerating fibres in the brain stem by the Marchi technique. They observed fibre degeneration in the superior colliculus and in the medulla below the

floor of the fourth ventricle. In 1951, Crosby & Woodburne traced the efferent descending pathways from the hypothalamus of the macaque by the Weil, toluidine blue and pyridine silver methods and demonstrated the existence of a medial and lateral projection.

With regard to the medial projection, Crosby & Woodburne (1951) demonstrated that in the medulla this pathway contributes fibres to the region of the dorsal nucleus of the vagus, the medial reticular formation and likely the nucleus ambiguus. Similar results have been obtained in the rat (Guillery, 1957).

Recently, using the Nauta-Gygax silver technique, the hypothalamus has been shown to project to the inferior olivary nucleus, the vestibular nuclei and the parahypoglossal area in the cat (Mabuchi & Kusama, 1970). However, the importance of this projection in the mediation of the cardiovascular responses elicited by hypothalamic stimulation is uncertain as destruction of this medial projection has recently been reported to have no effect on hypothalamic induced changes in heart rate and arterial pressure (McClure & Clark, 1968).

With regard to the lateral projection, until recently, this pathway was considered to terminate in the midbrain and rostral pons (Crosby & Woodburne, 1951). However in 1966, Cheatum & Matzke, using the Nauta technique demonstrated a projection to medullary nuclei in the cat. They found degenerating axons in the region of the dorsal nucleus of the vagus and in the nucleus medullae oblongatae centralis in the medullary reticular formation.

In 1965, Smith placed lesions in the hypothalamus of dogs, cats and rats at sites at which stimulation elicited an increase in heart

rate and arterial pressure and used the Nauta technique to reveal degenerating axons. Although it is uncertain whether the descending pathway involved was the medial or the lateral projection Smith demonstrated a projection to the inferior olivary nucleus, the lateral reticular nucleus and the nucleus intercalatus but failed to observe any degeneration in the dorsal motor nucleus of the vagus.

Information regarding the location of descending pathways from the hypothalamus has also been obtained by investigating the effect of lesions in the brain stem on the cardiovascular responses elicited by hypothalamic stimulation. Using this approach, Magoun, Ranson & Hetherington (1938) concluded that the descending pathway mediating the cardiovascular response elicited by hypothalamic stimulation in the cat occupied a large area of the midbrain as the response was not abolished by lesion of the medial longitudinal fasciculus and the periventricular grey. The following year, Wang & Ranson (1939b) placed lesions in the medulla of the cat and suggested that descending hypothalamic pathways involved in cardiovascular control were located in the lateral reticular formation of the medulla.

The recent demonstration (Manning, 1965) that destruction of the dorsolateral medullary reticular formation does not affect the pressor response elicited by hypothalamic stimulation prompts the suggestion that the hypothalamic pathway described by Wang & Ranson (1939b) is located in the ventrolateral region of the medullary reticular formation.

Recently, the site of termination of hypothalamo-medullary pathways has been investigated by recording the effect of hypothalamic stimulation on single unit activity in the medulla and the hypothalamus

has been shown to project to the parahypoglossal area of the dog (Alanis, Mascher & Miyamoto, 1966) and to the nucleus gigantocellularis in the medial reticular formation of the rat (Keene & Casey, 1970).

In summary, fibre and terminal degeneration has been observed in several medulla structures eg. inferior olivary nucleus, vestibular nuclei, dorsal nucles of the vagus, nucleus intercalatus, lateral reticular nucleus and parahypoglossal area after lesion of vasomotor areas in the hypothalamus. In addition, cardiovascular responses elicited by hypothalamic stimulation have been shown to be mediated by structures in the ventrolateral reticular formation of the medulla. Finally, a hypothalamic projection to reticular nuclei in the medial region of the medulla has been demonstrated. However, the failure to accurately localize the sites of stimulation and lesion in the hypothalamus and the lack of information concerning the function of the medullary nuclei receiving hypothalamic input precludes any conclusion regarding the role of these pathways in the control of the cardiovascular system.

B. Reflex neural control of the cardiovascular system

(a) Baroreceptor reflex

The study of reflex control of the cardiovascular system began with the demonstration that electrical stimulation of the aortic depressor nerve elicited decreases in heart rate and arterial pressure (Cyon & Ludwig, 1866). These authors suggested that the sensory endings of the depressor nerves were located in the heart and responded to changes in intracardiac pressure, but Köster & Tschermak (1903), demonstrated that the depressor nerves arose from the aortic arch and the roots of the great vessels and it was generally accepted by most workers at this time that changes in pressure in the aortic arch were the normal stimulus to the nerve fibres. Heymans & Ladon (1925) demonstrated the validity of this assumption by cross-circulation experiments: in a recipient dog they severed all structures connecting the head with the body except the vagosympathetic trunk and maintained the circulation in the isolated head by pumping blood from another dog. In this preparation they observed that pressure changes in the aorta of the recipient dog elicited bradycardia by a nervous mechanism involving the depressor nerve and the vagus.

In 1912 Sollmann & Brown observed that tugging the cephalic end of the common carotid artery produced bradycardia and arterial hypotension. Hering (1924) localized the origin of this reflex to the carotid bifurcation when he demonstrated that mechanical stimulation of the carotid sinus wall in the dog elicited bradycardia and arterial hypotension which was abolished by section of the branch of the gloss-

opharyngeal nerve that originated in the area of the carotid bifurcation.

With regard to baroreceptor hypotension the reflex vasodilation of blood vessels in sympathectomized dogs, cats and rabbits (Bayliss, 1902; Bishop, Heinbecker & O'Leary, 1933), was attributed by Rosenblueth & Cannon (1934) to the presence of efferent dilator fibres in the dorsal roots of spinal nerves and these vasodilator fibres were considered to be efferent pathway mediating baroreceptor induced hypotension. However, Thomas & Brooks (1937) demonstrated conclusively that the reflex changes in systemic arterial pressure elicited by alterations in baroreceptor activity were mediated by the vasoconstrictor fibres of the sympathetic nervous system as stimulation of the carotid sinus nerve failed to elicit any change in systemic arterial pressure after surgical extirpation of both sympathetic chains.

It is generally accepted that the baroreceptor reflex inhibits sympathetic vasoconstrictor activity, but recent evidence suggests that this inhibitory effect is not exerted on all central sympathetic structures as the postganglionic potentials evoked in the external carotid nerve during stimulation of a fast conducting sympathetic vasopressor pathway that originates in the lateral hypothalamus were not affected by baroreceptor activation (Gebber, Taylor & Weaver, 1973).

With regard to the efferent pathway mediating baroreceptor induced bradycardia Bronk, Ferguson & Solandt (1934) were the first to observe that increasing the pressure within the carotid sinus reduced the nervous activity in the sympathetic postganglionic nerves to the heart and that cutting both depressor nerves increased the spontaneous activity in the sympathetic nerves. This work was confirmed by

Downing & Siegel (1963) who also demonstrated that in the cat the cardiac rhythm observed in sympathetic nerves became random if the aortic depressor and carotid sinus nerves were sectioned. Further support for the inhibitory influence of the baroreceptors on the sympathetic input to the heart was obtained in the cat and dog by Green & Heffron (1966; 1968) and by Kezdi & Geller (1968). These authors recorded simultaneously from the carotid sinus and sympathetic postganglionic nerves and observed that the sympathetic activity varied inversely with baroreceptor discharge. In addition to inhibiting sympathetic activity, baroreceptor excitation is generally considered to increase the vagal input to the heart and according to most of the evidence available the bradycardia induced by baroreceptor stimulation is mediated by reciprocal changes in cardiac parasympathetic and sympathetic activity (Rosenblueth & Freeman, 1931; Wang & Borison, 1947; Gellhorn, 1964; Scher & Young, 1970; Thames & Kontos, 1970). However, this classical view has recently been challenged by Glick & Braunwald (1965) and by Robinson, Epstein, Beiser & Braunwald (1966). Glick & Braunwald suggested that the cardiac slowing elicited in dogs by increasing the arterial pressure involved predominantly vagal activity whereas the cardioacceleration elicited by decreasing the arterial pressure involved predominantly sympathetic discharge. Robinson et al found that in man the occurrence of a sympathetic or of a parasympathetic response depended on the pre-existing level of activity in these two limbs of the autonomic nervous system. More recently, Berkowitz, Scherlag, Stein & Damato (1969) concluded that, although reciprocal changes in sympathetic and parasympathetic tone did occur in the dog, baroreceptor control of heart

rate was due primarily to variations in sympathetic discharge and that changes in parasympathetic tone were of lesser importance.

In summary, the aortic arch and carotid sinus have been shown to contain receptors that respond to changes in arterial pressure. Activation of these baroreceptors was demonstrated to produce reflex vagal excitation and sympathetic inhibition resulting in bradycardia and arterial hypotension. However, the relative importance of the changes in vagal and sympathetic input to the heart during baroreceptor excitation is still uncertain.

(b) Chemoreceptor reflex

Heymans & Heymans (1927) were the first to demonstrate that in a dog connected to its head solely by the vagosympathetic trunk and supplied with blood from a donor dog, systemic hypoxia in the trunk of the recipient dog increased the respiratory activity in the isolated head as indicated by increased movements of the alae nasi and larynx and that this increased respiratory activity was abolished by bilateral vagotomy. Three years later, Heymans, Bouckaert & Dautrebande (1930) demonstrated that perfusion of the carotid bifurcation with hypoxic or hypercapnic blood produced hyperpnea and that this response was abolished by section of the ipsilateral carotid sinus nerve. In 1935 Zotterman recorded from the carotid sinus nerve and demonstrated conclusively that the increased frequency of impulses elicited during perfusion of the carotid bifurcation with hypoxic blood was due to activation of chemoreceptors and not of baroreceptors abnormally excited by the low oxygen tension in the perfusing fluid as suggested by Bogue & Stella (1935). In 1938, Comroe & Schmidt suggested that

the normal stimulus to chemoreceptors was a decrease in the oxygen tension of the blood since perfusion of the carotid body with blood containing a high concentration of carboxyhaemoglobin failed to elicit any change in respiratory rate. Their suggestion has been supported by Duke, Green & Neil (1952) and by Eyzaguirre & Lewin (1961a,b) who studied the effect of decreasing plasma oxygen tension on the impulse activity of chemoreceptor fibres in the carotid sinus nerve.

Many authors have reported that stimulation of the aortic and carotid chemoreceptors produces alterations in arterial pressure and heart rate. Bacq, Brouha & Heymans (1934) suggested that the hypertensive response to chemoreceptor stimulation was due to a reflex increase in sympathetic discharge to the blood vessels. A similar conclusion was reached by Dole & Morison (1940) but Bishop et al (1933) suggested that inhibition of parasympathetic nerves and dorsal root dilators also occurred during chemoreceptor induced hypertension. The failure to elicit any vasoconstriction or increase in arterial pressure during chemoreceptor stimulation in sympathectomized dogs even though the dorsal roots were intact (Bernthal, Motley, Schwind & Weeks, 1945) demonstrated conclusively that only sympathetic vasoconstrictor fibres were involved.

With regard to chemoreceptor induced changes in heart rate, Heymans, Bouchaert & Dautrebande (1931) found that local injection of cyanide into the arterial supply to the carotid body produced bradycardia. In spite of this observation, Asmussen & Chiodi (1941), Whitehorn, Edelmann & Hitchcock (1946) and Alveryd & Brody (1948) ascribed a peripheral chemoreceptor origin to hypoxic tachycardia.

Bernthal, Greene & Revzin (1951) stimulated the isolated carotid body with hypoxic blood in dogs breathing spontaneously or artificially respirated with different gas mixtures and observed that chemoreceptor stimulation produced bradycardia in artificially ventilated dogs and variable changes in heart rate in dogs breathing spontaneously. In 1958 Daly & Scott found that the magnitude of the bradycardia produced by stimulation of the carotid chemoreceptors in the dog was increased after lung denervation and concluded that hypoxic stimulation of peripheral chemoreceptors elicited primary reflex bradycardia that was partly or wholly masked by a secondary reflex tachycardia originating in the lungs. Scott (1966) obtained similar results in the cat and McQueen & Ungar (1966; 1971) demonstrated that the vagal bradycardia elicited by stimulation of the left or right carotid body in the artificially ventilated dog was mediated by both vagi. In 1956 Neil after allowing the characteristic response of tachycardia, hypertension and hyperpnea observed during systemic hypoxia to develop and to stabilize, perfused the carotid bifurcation with oxygenated Ringer-Locke solution: a fall in arterial pressure and a reduction in respiratory rate was observed during perfusion but no change in heart rate was observed, indicating a central origin of the tachycardia observed during systemic hypoxia. It appears therefore that chemoreceptor stimulation capable of eliciting reflex respiratory and vasomotor changes is not the cause of tachycardia observed during systemic hypoxia.

Many authors have reported that chemoreceptor stimulation produces an increase in ventilation. Recently, the chemoreceptors have been implicated in the ventilatory response observed during exercise

(Biscoe & Purves, 1967b). Carotid body chemoreceptors have been shown to be inhibited by efferent fibres in the carotid sinus nerve (Biscoe & Sampson, 1967; Fidone & Sato, 1970; Neil & O'Regan, 1971; Sampson & Biscoe, 1970) and to be excited by sympathetic activity in the cervical sympathetic nerve (Biscoe & Purves, 1967a; Mills & Sampson, 1969). As electrical stimulation of the cervical sympathetic nerves increases carotid chemoreceptor discharge (Biscoe & Purves, 1967a) and ventilation (Mills & Sampson, 1969) and passive movement of the hind limbs in man, dogs and cats elicits an increase in ventilation (Bilge, Velidedoglu, & Terzioglu, 1963; Comroe & Schmidt, 1943; Harrison, Harrison, Calhoun & Marsh, 1932; Honda & Minoguchi, 1957), cervical sympathetic activity and carotid chemoreceptor discharge (Biscoe & Purves, 1967b) the possibility exists that the increase in ventilation observed at the onset of exercise (Dejours, 1959; 1964) may be mediated by increasing chemoreceptor discharge which is known to increase ventilation (Comroe & Schmidt, 1938; Daly & Scott, 1958; Heymans et al, 1930; Neil, 1956; Scott, 1966).

In summary, the aortic body and carotid body have been shown to contain receptors which respond to changes in the chemical composition of the blood. The normal stimulus to these chemoreceptors is a decrease in oxygen tension of the plasma. Activation of the chemoreceptors produces an increase in arterial pressure due to sympathetic excitation, bradycardia primarily due to vagal inhibition and increased ventilation due to an increase in the respiratory rate and tidal volume. Although the functional significance of the bradycardia is uncertain as systemic hypoxia produces tachycardia, a functional

role of chemoreceptor induced hyperpnea in exercise has been suggested recently (Biscoe & Purves, 1967b).

(c) Projection of baroreceptor and chemoreceptor input in the central nervous system

In 1961 Smith & Pearce, recorded single units in the cat medulla which exhibited spontaneous activity in phase with the heart beat and found that such units were only located in the region of the nucleus of tractus solitarius. Similar results have been observed by Hellner & Baumgarten (1961), by Salmoiraghi (1962) and by Middleton & Woolsey (1965). The possibility that the pattern of discharge of these units was due to baroreceptor or chemoreceptor input was suggested by the demonstration of degenerating fibres in the nucleus and tractus solitarius after section of the IX th and X th cranial nerves (Cottle, 1964). Additional support for this possibility is the recording of compound action potentials in the carotid sinus and aortic depressor nerves evoked antidromically by electrical stimulation of the nucleus of tractus solitarius (Crill & Reis, 1968) and the recording of field potentials in the nucleus of tractus solitarius during electrical stimulation of the carotid sinus nerve in the cat (Sampson & Biscoe, 1968; 1970b; Seller & Illert, 1969; Miura & Reis, 1969). Finally, Humphrey (1967) demonstrated that stimulation of the carotid sinus nerve in the cat produced multi- and single-unit potentials in the medial portions of the nucleus of tractus solitarius and that destruction of this area abolished both the reflex decrease in arterial pressure and the vagally mediated bradycardia produced by distension of the carotid sinus.

Recently, other medullary structures have been shown to receive cardiovascular afferent fibres. Short and long latency evoked potentials have been recorded from an area of the medial reticular formation corresponding to the location of the paramedian reticular nucleus (Humphrey, 1967). Because of the latencies of the evoked potentials recorded in the medial reticular formation and the demonstration that the amplitude of the evoked potentials could be changed by varying the frequency of the stimulus applied to the nucleus of the tractus solitarius and the carotid sinus nerve, Humphrey suggested that carotid sinus nerve fibres relayed to the medial reticular formation either directly or through the nucleus of tractus solitarius. Similar results have been obtained by Seller & Illert (1969) and by Miura & Reis, (1969). Short latency evoked potentials have also been recorded in the nculeus medullae oblongatae centralis (Miura & Reis, 1969; Seller & Illert, 1969) indicating a direct projection of carotid sinus nerve fibres to this area. Similarly, a direct projection of cardiovascular afferent fibres to the paramedian reticular nucleus, nucleus medullae oblongatae centralis, nucleus gigantocellularis and nucleus cuneatus medialis and lateralis (Crill & Reis, 1968) has been demonstrated by recording compound action potentials in the carotid sinus and aortic depressor nerves that were evoked antidromically by electrical stimulation of these medullary structures.

It is well documented that cardiovascular afferent fibres project to medullary structures, but attempts to demonstrate baroreceptor and chemoreceptor input to the hypothalamus have yielded equivocal results. Hilton and Spyer (1968) recorded single unit

activity in the hypothalamus during baroreceptor stimulation and reported that application of static pressure pulses to the carotid sinus of the cat altered the spontaneous activity of eight hypothalamic units. Similar results have been obtained in an extensive investigation by Spyer (1972). However, the demonstration that single units in the hypothalamus of the cat responded to changes in arterial pressure after baroreceptor denervation and pontine transection (Baust & Katz, 1961; Baust, Niemczyk, Schaeffer & Vieth, 1962; Frazier, Taquini, Boyarski, & Wilson, 1965) and as the method used by Hilton & Spyer to excite baroreceptors is known to elicit arterial hypotension (Heymans & Neil, 1957), the possibility exists that the results obtained by Hilton and Spyer were due to the direct effect of arterial pressure changes on the hypothalamic units. Similarly, the evidence for a chemoreceptor input to the hypothalamus is uncertain as the response of hypothalamic units in the rat to changes in arterial PO_2 and PCO_2 have been attributed to chemosensitivity of these neurones (Cross, 1964; Cross & Silver, 1963) and to input from chemoreceptors in the brain stem (Cross & Dyer, 1971).

In summary, it is well documented on the basis of anatomical and electrophysiological data that baroreceptor and chemoreceptor fibres project to discrete medullary nuclei (i.e. nucleus of tractus solitarius, paramedian reticular nucleus and nucleus medulla oblongatae centralis). However, attempts to demonstrate cardiovascular afferent input to the hypothalamus have yielded equivocal results and the existence of this input is uncertain.

C. Central neural regulation of cardiovascular reflexes

During the past decade, electrical stimulation and ablation of many structures in the central nervous system has been shown to alter the reflex changes in heart rate and arterial pressure elicited by excitation of baroreceptors and chemoreceptors.

With regard to the cerebellum, Moruzzi (1940) using decerebrate vagotomized cats demonstrated that both the pressor response elicited by carotid occlusion and the depressor response produced by electrical stimulation of the aortic depressor nerve were inhibited by electrical stimulation of the vermis cerebelli. He suggested that the cerebellum exerted an inhibitory influence on the medullary and pontine structures involved in the baroreceptor reflex. This suggestion has been supported by the findings of Reis & Cuénod (1965) who observed that both the pressor response produced by carotid occlusion and the depressor response produced by distension of the carotid sinus were augmented after cerebellectomy. In addition Achari & Downman (1970) demonstrated in cats that electrical stimulation of the fastigial nucleus of the cerebellum inhibited the reflex decrease in heart rate elicited by stimulation of the carotid sinus nerve. This effect was shown to be due to inhibition of the vagal component of the baroreceptor reflex as the inhibitory response was not affected by spinal transection (Hockman, Livingston & Talesnik, 1970).

With regard to the medulla, stimulation of the inferior olivary nucleus in cats has been reported to inhibit the bradycardia and arterial hypotension elicited by stretching the carotid sinus (Smith & Nathan, 1966). However, the suggestion that stimulation of the inferior

olivary nucleus inhibits baroreceptor induced hypotension is equivocal as the decrease in the magnitude of the arterial hypotension may have been due to the decrease in the magnitude of the bradycardia. Recently, in spinal cats, electrical stimulation of the paramedian reticular nucleus in the medial reticular formation of the medulla has been shown to inhibit the reflex vagal bradycardia elicited by electrical stimulation of the aortic depressor nerve (Calaresu & Thomas, 1971) and by intravenous administration of noradrenaline (Klevans & Gebber, 1971).

Hilton (1963) was the first to demonstrate that the hypothalamus could influence cardiovascular reflexes. He observed in the cat that electrical stimulation of the hypothalamus decreased the magnitude of the bradycardia and arterial hypotension elicited by stimulation of the carotid sinus nerve. However, his conclusion that reflex hypotension was inhibited during central stimulation is equivocal as the possibility exists that the decrease in the magnitude of the hypotension may have been due to the inhibition of reflex cardiac slowing. This possibility is supported by the recent demonstration that hypothalamic stimulation inhibits the vagal component of the baroreceptor reflex (Djojosugito, et al, 1970; Gebber & Snyder, 1970; Hockman & Talesnik, 1971; Humphreys, Joels & McAllen, 1971) but not the sympathetic component (Humphreys & Joels, 1972). Finally, the excitatory effect of carotid sinus nerve stimulation on single unit activity in the nucleus of tractus solitarius has been shown to be inhibited during electrical stimulation of the dorsomedial hypothalamus (Adair & Manning, 1973). Although the mechanism of this hypothalamic

inhibition of cardiovascular reflexes is unknown, primary afferent depolarization of carotid sinus nerve fibres in the nucleus of tractus solitarius has been observed during hypothalamic stimulation (Weiss & Crill, 1969).

Limbic structures have also been shown to exert an influence on cardiovascular reflexes. Stimulation of the anterior hypothalamus, preoptic area, amygdala and septum in cats has been shown to increase the magnitude of the reflex vagal bradycardia elicited by intravenous administration of noradrenaline (Klevans & Gebber, 1970). Electrical stimulation of the central grey in the midbrain and of the ventral hippocampus has been reported to inhibit or to augment the reflex vagal bradycardia elicited by stimulation of the carotid sinus nerve (Hockman, Talesnik & Livingston, 1969).

To conclude, evidence has been presented which demonstrates that excitation or ablation of structures within the central nervous system alter the reflex cardiovascular responses elicited by the baroreceptors and chemoreceptors. However the locations and mechanisms involved in the interaction are unknown.

D. Conclusions and statement of objectives of experimental work

A review of the literature concerning the role of the medulla and hypothalamus and of baroreceptor and chemoreceptor reflexes in the control of the cardiovascular system has been presented. It is well documented that the medulla contains vasomotor and cardioregulatory areas and mediates reflex changes in heart rate and arterial pressure. However, the function of discrete medullary structures in the control of the cardiovascular system is uncertain due to the failure to accurately localize the sites of stimulation and recording. Similarly, many investigations have shown that stimulation or ablation of the hypothalamus produces marked changes in heart rate and arterial pressure but the precise location of hypothalamic vasomotor areas is unknown.

The interaction of central neural structures with the baroreceptor reflex is well documented but the location of these structures, and the sites and mechanism(s) involved in this interaction are uncertain. In addition, the possibility that a similar interaction with the chemoreceptor reflex has not been investigated.

In view of these uncertainties, the aim of this project was to study the role of a well defined hypothalamic region in the control of cardiovascular reflexes and to investigate the mechanism(s) involved in this control. In the first series of experiments, attempts were made to localize a discrete vasomotor region in the hypothalamus and to investigate the possibility that baroreceptor and chemoreceptor inputs project to this hypothalamic region. In the second series, the effect of stimulation of this region on the cardiovascular response elicited by baroreceptor and chemoreceptor stimulation was determined.

To investigate the mechanism of the hypothalamic interaction with cardiovascular reflexes, the medullary structures involved in mediating these reflexes were identified in the third series of experiments and in the fourth series, the effect of hypothalamic stimulation on the neural activity in these medullary structures was determined.

As the experimental work done in this investigation involved several related projects, a brief summary of the literature relevant to each project and the rationale for doing the work presented in this thesis will be discussed.

(a) Investigation of cardiovascular afferent inputs to the hypothalamus

It is generally accepted that the reflex changes in heart rate and arterial pressure elicited by stimulation of cardiovascular afferent fibres are mediated by altering the level of activity in the vasomotor and cardioregulatory areas located in the pons and medulla (Uvnäs, 1960). This view is supported by the observation that many pontomedullary areas from which changes in heart rate and arterial pressure are elicited by electrical stimulation (Achari, Downman & Weber, 1968; Alexander, 1946; Calaresu & Pearce, 1956; Calaresu & Thomas, 1971) receive cardiovascular afferent fibres from the ninth and tenth cranial nerves (Biscoe & Sampson, 1970; Cottle, 1964; Crill & Reis, 1968; Humphrey, 1967; Miura & Reis, 1969; Seller & Illert, 1969). It has also been suggested that baroreceptor and chemoreceptor reflexes may be mediated by altered activity in vasomotor and cardioregulary areas located in the hypothalamus. (Hilton &

Spyer, 1971; Peiss, 1965). Although it is well documented that stimulation or ablation of the hypothalamus produce marked changes in heart rate and arterial pressure (Hilton, 1963; Keller, 1960; Korner, 1971) the precise location of vasomotor areas within the hypothalamus is unknown because of the poor localization of the sites of stimulation. In addition, attempts to demonstrate baroreceptor and chemoreceptor input to the hypothalamus have yielded equivocal results. Hilton and Spyer (1968) have reported that in the cat the spontaneous activity of eight hypothalamic units was altered by the application of static pressure pulses to the carotid sinus. However, as it has been shown that increased pressure in the carotid sinus elicits systemic hypotension (Heymans & Neil, 1958) and that single units in the hypothalamus of cats respond to the changes in arterial pressure induced by carotid occlusion and by injection of adrenaline before and after baroreceptor denervation and pontine transection (Baust & Katz, 1961; Baust, Niemczyk & Schaeffer, 1962; Frazier, Taquini, Boyarski & Wilson, 1965) the possibility exists that the results obtained by Hilton and Spyer (1968) were due to the direct effect of arterial pressure changes on the hypothalamic units. Similarly, the existence of an input to the hypothalamus from peripheral chemoreceptors is also uncertain as the response of hypothalamic units in the rat to changes in arterial P_{O_2} and P_{CO_2} has been interpreted as being due to chemosensitivity of these neurones (Cross, 1964; Cross & Silver, 1963) and to an input from chemoreceptors in the brain stem (Cross & Dyer, 1971).

The purpose of the experiments reported here was to determine whether or not baroreceptor and chemoreceptor afferent inputs project

to the hypothalamus and alter the neural activity in localized hypothalamic vasomotor areas. The first objective was to localize a discrete region in the hypothalamus, electrical stimulation of which produced reproducible changes in heart rate and arterial pressure. The second objective was to record the activity of single units in this region during electrical stimulation of the carotid sinus nerve (CSN). To ensure that the responses of single units were not due to changes in blood flow to the hypothalamus the CSN was stimulated at frequencies such that reflex bradycardia and arterial hypotension were not observed. The final objective was to demonstrate the specificity of the input to hypothalamic units by comparing their response during electrical stimulation of baroreceptor and chemoreceptor fibres in the CSN to that elicited by selective stimulation of either baroreceptors or chemoreceptors.

(b) Investigation of the role of the hypothalamus in the mediation of cardiovascular reflexes

It is well documented that the primary response to carotid body chemoreceptor excitation in the dog and in the cat consists of bradycardia, arterial hypertension and hyperventilation (Bernthal, Green & Revzin, 1951; Bernthal, Motley, Schwind & Weeks, 1945; Comroe & Mortimer, 1964; Daly & Scott, 1958, Kontos, Vetrovec, & Richardson, 1970; MacLeod & Scott, 1964; McQueen & Ungar, 1971), and that the primary reflex bradycardia may be obscured by the cardioacceleration secondary to the chemoreceptor induced hyperventilation (Daly & Scott, 1958; Scott, 1966).

Experiments designed to elucidate the peripheral mechanism mediating the cardiac slowing have yielded conflicting results. Evidence has been presented suggesting that carotid body chemoreceptor induced bradycardia is mediated by both an increase in vagal input and a decrease in sympathetic input to the heart (Daly & Scott, 1958; Downing, Remensnyder & Mitchell, 1962; MacLeod & Scott, 1964). On the other hand, the demonstration that intravenous administration of atropine or bilateral cervical vagotomy consistently abolishes the reflex bradycardia elicited by chemoreceptor stimulation (McQueen & Ungar, 1971) supports the contention that the reflex cardiac slowing may be mediated exclusively by the vagus. Additional evidence in support of this latter possibility is the failure to demonstrate a significant change in the electrical activity recorded from the inferior cardiac nerve of the cat during perfusion of the carotid sinus with hypoxic blood (Downing & Siegel, 1963).

There is a similar uncertainty concerning the role of supra-bulbar nervous structures in the control of chemoreceptor induced bradycardia. The demonstration that the bradycardia observed during systemic hypoxia in the thalamic rabbit is abolished by decerebration (Korner, Uther & White, 1969) has been considered as evidence that the hypothalamus is the central structure mediating carotid body chemo-receptor bradycardia. However, this conclusion is not unequivocal as it is possible that systemic hypoxia may stimulate directly the central nervous system as well as excite peripheral chemoreceptors, as suggested by the demonstration that hypoxia may elicit a decrease in heart rate in rabbits after section of the carotid sinus and aortic depressor

nerves (Korner et al, 1969).

In view of the apparent discrepancies concerning the peripheral mechanism and the central control of chemoreceptor bradycardia three series of experiments were done. In the first series the quantitative changes in the cardiovascular responses to carotid body chemoreceptor stimulation before and after selective elimination of either the sympathetic or of the parasympathetic input to the heart were determined. In the second, the role of suprabulbar structures in the control of chemoreceptor bradycardia was assessed by comparing the cardiovascular responses to selective excitation of carotid body chemoreceptors before and after midcollicular decerebration. In the third, the effect of discrete electrical stimulation of the posteromedial hypothalamus on the chemoreceptor induced changes in heart rate was investigated.

(c) Identification of medullary structures mediating reflex vagal bradycardia

It is well documented that reflex cardiovascular changes elicited by stimulation of baroreceptors and chemoreceptors are mediated at the level of the medulla (Korner, Uther & White, 1969; Lindgren, 1961; Reis & Cuénod, 1965; Wang & Chai, 1967). However the precise location of the cardioinhibitory neurones involved in the vagal component of these reflexes is uncertain as electrical stimulation of the dorsal nucleus of the vagus (DX), generally considered to be the site of origin of cardioinhibitory neurones, does not produce bradycardia in the cat (Achari, Downman & Weber, 1968; Borison & Domjan, 1970; Calaresu & Pearce, 1965b; Gunn, Sevelius, Puiggari, & Myers, 1968).

Additional evidence that the DX does not possess a cardioinhibitory function is the demonstration that destruction of this nucleus does not abolish the vagal cardioinhibitory response to brain stem ischemia in the acute preparation (Borison & Domjan, 1970), or the bradycardia elicited by electrical stimulation of the cut peripheral vagus in the chronic preparation (Kerr, 1969).

Although it has been shown that electrical stimulation of certain medullary nuclei elicits cardiac slowing it is not possible on the basis of the evidence in the literature to conclude that vagal cardioinhibitory neurones are located in these structures. Electrical stimulation of the nucleus of tractus solitarius (NTS) has been shown to elicit vagal bradycardia (Achari et al, Calaresu & Pearce, 1965b; Gunn et al, 1968; Quest & Gebber, 1972; Seller & Illert, 1969). However, this bradycardia is probably due to stimulation of primary afferent fibres as baroreceptors and chemoreceptors are known to terminate in the NTS (Biscoe & Sampson, 1970; Cottle, 1964; Crill & Reis, 1968; Humphrey, 1967; Miura & Reis, 1969; 1972; Seller & Illert, 1969). Similarly, uncertainty exists concerning the bradycardia elicited from the region of the nucleus ambiguus (NA) as the type of stimulus used appears to be important. Heating the reticular formation in the region of the NA (Chai, Mu & Brobeck, 1965), advancing the electrode into the region (picure, Gunn et al, 1968) or electrical stimulation with bipolar electrodes (Gunn et al, 1968; Quest & Gebber, 1972) elicits vagal bradycardia. On the other hand it has been reported that electrical stimulation in the region of the NA with unipolar electrodes fails to elicit any changes in heart rate (Chai et al, 1965). It has

also been shown that electrical stimulation of the nucleus gigantocellularis located in the medial reticular formation of the medulla elicits vagal bradycardia (Achari et al, 1968); however, this structure was not considered to be the site of origin of cardioinhibitory neurones but part of a descending cardioinhibitory pathway originating in the limbic cortex.

In view of the paucity of information concerning the site of origin of cardioinhibitory neurones this portion of the investigation involved a systematic exploration of the medulla for vagal cardioinhibitory sites with histological localization of the responsive sites. Spinal animals were used to ensure that evoked changes in heart rate were mediated only by the vagus. In addition, in some animals ipsilateral and then contralateral vagotomy were done to determine whether the bradycardia was mediated by crossed or uncrossed pathways. Finally, to ascertain whether the cardiac slowing elicited during medullary stimulation was due to excitation of afferent fibres and interneurones involved in reflex vagal bradycardia rather than to direct stimulation of efferent cardioinhibitory neurones, the effect of administration of a barbiturate on the vagal cardiac slowing elicited from various medullary sites was investigated. This agent was used as it has been shown to depress synaptic transmission of baroreceptor and chemoreceptor inputs to the medulla (Miura & Reis, 1969).

(d) <u>Identification of medullary sites mediating hypothalamic inhibitation of reflex vagal bradycardia</u>

The role of the medulla oblongata in the mediation of reflex

cardiovascular changes elicited by stimulation of baroreceptors and chemoreceptors is well established (Lingren, 1961; Reis & Cuénod, 1965; Wang & Chai, 1967). However, the location of different components of these reflex arcs and particularly of cardioinhibitory neurones is uncertain (Achari, Downman & Weber, 1968; Borison & Domjan, 1970; Calaresu and Pearce, 1965b; Gunn, Sevelius, Puiggari & Meyers, 1968; Kerr, 1969) although recent investigations in the cat have implicated the nucleus ambiguus as the site of origin of cardioinhibitory neurones (Borison & Domjan, 1970; Gunn et al, 1968; Kerr, 1969).

With regard to the role of suprabulbar structures in the mediation of cardiovascular reflexes, it has been demonstrated during the past decade that the hypothalamus can exert an influence on ongoing cardiovascular reflexes mediated by the lower brain stem. In particular, electrical stimulation of the hypothalamus has been shown to inhibit the vagal bradycardia (Djojosugito, Folkow, Kylstra, Lisander & Tuttle, 1970; Gebber & Snyder, 1970; Hilton, 1963; Hockman & Talesnik, 1971; Humphreys, Joels & McAllen, 1971) and the arterial hypotension (Hilton, 1963; Gebber & Snyder, 1970) elicited by baroreceptor excitation. However, information regarding discrete inhibitory regions in the hypothalamus is very scanty as in many of these investigations there was no precise localization of sites of stimulation.

As the inhibitory function of the hypothalamus on cardiovascular reflexes mediated at the level of the medulla is well established, but the precise location of the medullary sites at which the hypothalamic influence takes place is uncertain, attempts were made in the final project of the investigation to localize medullary structures involved

in the vagal component of baroreceptor and chemoreceptor reflexes which could be inhibited by hypothalamic stimulation. Two series of experiments were done: in the first, the effect of electrical stimulation of the hypothalamus on the magnitude of the vagal bradycardia produced by stimulation of the medulla was determined; in the second, attempts were made to record field potentials in these medullary regions during stimulation of the hypothalamus.

METHODS

A. General procedures

Results were obtained in 131 cats (1.9 - 3.7 kg) anaesthetized with alpha-chloralose (60 mg/kg) after ethyl chloride and ether induction. The trachea, femoral artery and femoral vein were cannulated routinely. Arterial pressure, monitored by a Statham transducer connected to a catheter in the left femoral artery, heart rate, computed by a Grass 7P4 tachograph preamplifier, and respiratory frequency, monitored as a change in temperature of tidal air by a thermistor in the tracheal cannula were recorded on a Grass 7 polygraph. The femoral vein was used to inject drugs. The animals were placed in the prone position with the heads fixed in a Kopf stereotaxic instrument. The rectal temperature of the animals was kept at 37°C ± 0.2 by a heating pad controlled by a Yellow Springs 73 temperature controller.

B. Surgical procedures

To expose the caudal portion of the floor of the fourth ventricle the occipital bone was removed and the cut edges were packed with Horsley's bone wax to prevent bleeding. The cerebellum was totally removed by suction and warm mineral oil was poured over exposed nervous tissue to prevent drying.

To stimulate and record in the hypothalamus, the brain was exposed by bilateral parietal craniotomy. In selected experiments, the effect of decerebration on various experimental manoeuvers was determined.

The decerebration done through a hole bored in the temporal and parietal bone on the left side of the skull consisted of transection of the neuraxis at the intercollicular level using a blunt spatula and subsequent removal of the nervous tissue rostral to the transection. The left or right carotid sinus nerve (CNS) was exposed by a lateral approach; the submaxillary gland and a segment of the hypoglossal nerve near the carotid bifurcation were removed, and the CSN was isolated from surrounding connective tissue and crushed near its site of origin at the carotid bifurcation. In selected experiments the CSN was isolated in such a way as to leave the blood supply to the carotid body intact and the medial thyroid artery was cannulated for subsequent infusions.

C. Electrical stimulation

(a) Stimulation of the hypothalamus

Unipolar stainless steel electrodes (tip diameter 20-50 μm, shaft diameter 175 μm, insulated with Insl-X, Insl-X Corp, Yonkers, N.Y. to approximately 150 μm from the tip) were used to stimulate the hypothalamus. The DC resistance of the electrodes in saline was 40-50 kilohms. The indifferent electrode was an alligator clip attached to exposed scalp muscle. To investigate the effects of hypothalamic stimulation on heart rate and arterial pressure and on medullary and reflex vagal bradycardia the parameters of stimulation were 4-8 v, (70-200 μA) 40-60 Hz, 0.2-0.5 msec for 10-20 sec. To elicit field potentials in the medulla, the hypothalamus was stimulated at 4-8 v,

using single or twin pulses (interval 5-10 msec) of 0.2-0.5 msec duration at a frequency of 0.5 Hz. The stimulus was generated by a Grass S44 stimulator and was delivered to the hypothalamus through a Grass (SIU5) stimulus isolation unit. On each penetration, the site of stimulation selected was that yielding the maximum response.

(b) Stimulation of the medulla

In preliminary experiments the right side of the medulla was explored for cardioinhibitory sites in a region extending from 3 mm caudal to 7 mm rostral to the obex and 5 mm lateral to the midline. It was found that cardioinhibition could be most readily elicited between 1 mm caudal and 4 mm rostral to the obex and 5 mm lateral. This area coincides with the region described anatomically (Cottle, 1964) and electrophysiologically (Calaresu & Pearce, 1965; Crill & Reis, 1968) as the site of termination of primary cardiovascular afferents and therefore systematic exploration was restricted to this region. The reference point for positioning the electrode was the obex and the region was explored systematically on a grid with points of penetration one millimeter apart. Unipolar stainless steel electrodes were used (tip diameter 5 - 10 μm, shaft diameter 175 μm, insulated with Insl-X, to within 75 μm from the tip). The DC resistance of the electrodes in saline was 40 - 60 kilohms. The indifferent electrode was an alligator clip attached to exposed scalp muscle. The stimulus used consisted of a 15 to 30 second train of rectangular pulses (4-8 v, 20-40 Hz, 0.2 msec). These parameters of stimulation were found to elicit optimal cardiac slowing.

(c) Stimulation of the carotid sinus nerve (CSN)

The CSN was placed on bipolar stainless steel electrodes, the skin edges of the wound were sutured to the stereotaxic frame and the nerve was covered with mineral oil at 37°C. Stimuli to the CSN were 0.1 - 0.5 msec pulses from a Grass S4 stimulator through a Grass SIU5 stimulus isolation unit. The "threshold" stimulus was defined as the voltage required to elicit a 20% decrease in heart rate using a 10 sec stimulus train of 50 Hz and 0.2 msec pulse duration. The CSN was then repetitively stimulated at 0.1 to 0.5 Hz at stimulus intensities of 2 to 5 times threshold while the hypothalamus was systematically explored for responsive units or the CSN was stimulated at 20 to 60 Hz (4-10 v, 0.1 to 0.5 msec) and the effect of simultaneous stimulation of the hypothalamus on the CSN induced cardiac slowing was determined.

(d) Stimulation of the cervical vagus nerve

In selected experiments the peripheral end of the left cut vagus was stimulated. The left vagus, exposed by a lateral approach, was placed on bipolar stainless steel electrodes, the skin edges of the wound were sutured to the stereotaxic frame and the nerve was kept in a pool of mineral oil at 37°C. The parameters of stimulation were 2 - 6v, 20 - 40 Hz, 0.2 msec for 15 - 30 sec.

D. Selective stimulation of baroreceptors and chemoreceptors

The chemoreceptors of the right carotid body were stimulated by injection of sodium cyanide (NaCN, British Drug Houses, Toronto,

Canada, 50 - 100 µg in 0.05 - 0.10 ml of a 1 mg/ml solution in saline) into the right common carotid artery via a cannula in the medial thyroid artery; this method of administration has been shown to excite selectively the carotid body chemoreceptors. (Fidone & Sato, 1969; Jacobs, Sampson & Comroe, 1971). The appearance of marked transient bradycardia or arterial hypertension or polypnea or a combination of these responses after administration of NaCN was considered evidence of chemoreceptor excitation. A control injection of saline (0.05 - 0.1 ml) into the common carotid artery did not elicit any cardiovascular or respiratory responses.

Baroreceptors were stimulated by intravenous administration of noradrenaline (NA, 0.5 - 2.0 µg/kg); this method has been shown to selectively excite baroreceptors (Gebber & Snyder, 1970). The appearance of a marked transient bradycardia after administration of NA was considered evidence of stimulation of baroreceptors.

E. Recording neural activity in the hypothalamus and in the medulla

(a) Single unit recording

Stainless steel microelectrodes (Green, 1958) with a tip diameter of 1 - 3 µ and a resistance in saline of 0.5 to 3 MΩ were used for extracellular recording from single units in the hypothalamus. The indifferent electrode was a needle inserted into the brain substance through a small hole in the skull. Signals were led into a Grass P15 differential preamplifier with a bandpass of 300-3000 Hz. The amplifier output was connected to a Tektronix 565 oscilloscope for observation and photography. The signals were also stored on a

Philips Analog 7 tape recorder for further analysis.

(b) Recording of field potentials

Concentric bipolar electrodes (SNEX 100, Kopf Instruments, Tujunga, Calif., tip separation 500 μm, DC resistance in saline 40-50 kilohms) were used to record field potentials in the medulla during hypothalamic stimulation. Signals were led into a Grass P15 differential preamplifier with a bandpass of 0.3-3000 Hz and the amplifier output was connected to Tektronix 564 and 565 oscilloscopes for observation and photography; the signals were also stored on a Philips Analog 7 tape recorder for further analysis.

F. Investigation of efferent pathways mediating cardiovascular responses

To investigate whether the changes in heart rate and arterial pressure elicited by electrical stimulation of the hypothalamus, medulla and CSN and by selective excitation of carotid chemoreceptors were mediated through changes in sympathetic or parasympathetic activity, the effect of blocking either output on the response to further stimulation was investigated. The parasympathetic input to the heart was abolished by bilateral cervical vagotomy and the sympathetic input was blocked by one of two methods. The first method was the intravenous administration of the beta-adrenergic blocking agent propranolol (1.5 mg/kg, Inderal, Ayerst Laboratories, Montreal); doses of 0.75 mg/kg of this drug in chloralosed cats have been shown to abolish the cardioacceleration produced by electrical stimulation of the right stellate ganglion (Black, Duncan & Shanks, 1965). The second

method was transection of the spinal cord at C_7; spinal transection at this level in the cat usually does not abolish spontaneous respiration and is rostral to the site of origin of spinal sympathetic neurones (Henry & Calaresu, 1972). However, in several animals artificial ventilation was required and was administered with an E & M V5KG respirator.

In addition, in selected experiments, to ensure that the changes in heart rate elicited by stimulation of the medulla and reflex excitation of baroreceptors were mediated solely by the vagus nerves, and to investigate the effects of hypothalamic stimulation on this vagal response without altering the sympathetic input to heart and blood vessels, the spinal cord was cut at C_7.

G. Histological localization of sites of recording and of stimulation in the central nervous system

The hypothalamic stimulating and recording electrodes were positioned with reference to the stereotaxic coordinates obtained from the atlas of Jasper and Ajmone Marsan (1954). The reference point for positioning medullary stimulating and recording electrodes was the obex. To obtain accurate anatomical localization of the electrode tip the sites of recording and stimulation were marked by depositing iron from the tip of the electrode (5 μA for 20-30 sec. electrode tip positive). The animals were perfused with 0.9% saline followed by a solution of 1% potassium ferrocyanide in 10% formalin. The brain was fixed for 3-7 days and 50 μ frozen sections were cut and stained with thionin. The typical appearance of an iron deposit at a site of recording

in the hypothalamus is shown in Figure 1 and the appearance of an iron deposit at a site of stimulation in the medulla is shown in Figure 2.

H. Analysis of data

(a) Cardiovascular response

Heart rate was computed in beats/minute from a count of the systolic peaks of the arterial pressure record in the 20 seconds before electrical stimulation and injection of drugs and in the last 10 seconds of the train of stimulation of the hypothalamus, medulla, CSN and vagus and in a 5 second period during the maximum cardiac response elicited by injection of sodium cyanide and noradrenaline. Mean arterial pressure (diastolic pressure pulse and third of the pulse pressure) was measured before the stimulus and injection and at highest or lowest level elicited by electrical stimulation or injection of drugs. The differences in heart rate and mean arterial pressure between control and stimulation or injection values were averaged from three runs. A change in heart rate was considered to be present only if changes of 6 or more beats/min were elicited by electrical stimulation or by injection of drugs. Changes in heart rate elicited by electrical stimulation and by injection of drugs before and after vagotomy, administration of propranolol, spinal transection or decerebration, and before and during hypothalamic stimulation were similarly computed. Mean values (rounded to the nearest integer) of changes in heart rate and arterial pressure for each group were then compared.

Student's "t" test for correlated samples were used for

Figure 1

Transverse section of the diencephalon with an iron deposit (at the arrow) corresponding to the tip of a recording electrode in the posteromedial hypothalamus. 50 um section, thionin and potassium ferrocyanide stain. Plane of section at approximately F 10. Fx, fornix; Hp, posterior hypothalamus; Hd, dorsal hypothalamus; MT, mammillo-thalamic tract. Calibration 1 mm.

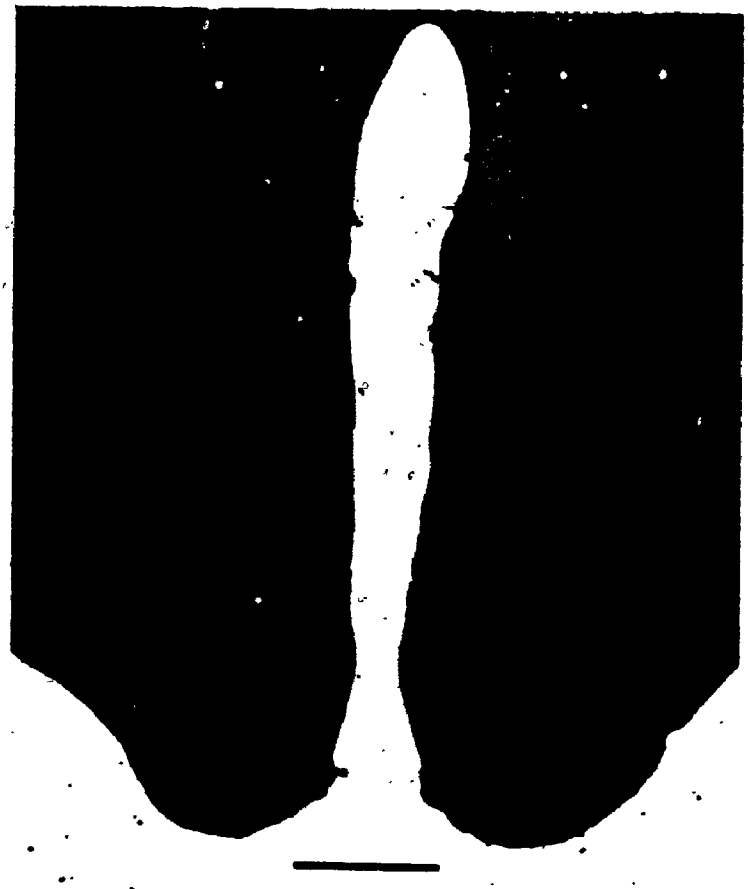


Figure 2

Transverse section of the medulla of a cat approximately 1.5 mm rostral to the obex with an iron deposit (at the arrow) corresponding to the tip of a stimulating electrode in the nucleus ambiguus. 50 um section, thionin and potassium ferrocyanide stain, calibration 1 mm.

statistical analysis; a probability of less than 0.05 that the difference between two means could occur by chance was considered significant.

(b) Single unit activity

To investigate the effects of electrical stimulation of the carotid sinus nerve (CSN) on hypothalamic unit activity, a Biomac 1000 (Data Instruments Ltd. London) signal analyzer was used to generate post-stimulus time (PST) histograms. A decrease in the probability of firing of a neurone measured by the PST histogram compared to the control probability was designated "inhibition". Increased probability was designated "excitation". In slowly firing cells (2 spikes/sec) at least 50 summated sweeps were usually needed to demonstrate inhibition. The latency of the responses observed has been arbitrarily defined as the time from the stimulus artifact to the appearance of a change in the probability of firing of a neurone. The duration of the responses was considered to be the time during which the probability of firing of a neurone was altered from the control probability by stimulation of the CSN.

RESULTS

A. Cardiovascular response elicited by stimulation of the posteromedial hypothalamus

A systematic exploration of the hypothalamus in a region extending from 8.5 mm rostral to 13 mm rostral to the inter-auricular line, from the midline to 3 mm lateral and from 10 mm dorsal to 3 mm dorsal to the inter-auricular line was done to find vasomotor areas that might be potential sites of termination of baroreceptor or chemoreceptor input. It was found that stimulation of a localized area in the posteromedial hypothalamus produced marked changes in heart rate and arterial pressure. Selective electrical stimulation of 20 histologically localized sites in the posteromedial hypothalamus in nine animals consistently elicited cardioacceleration and arterial hypertension. The mean changes in heart rate and arterial pressure elicited by electrical stimulation of these sites are presented in Table 1 and the location of these sites is shown in Figure 3.

To investigate whether the effects on heart rate induced by electrical stimulation of this hypothalamic area were mediated by changes in activity of the sympathetic or of the parasympathetic input to the heart, the responses before and after blocking either input were compared. In three animals, bilateral vagotomy did not alter the cardioacceleration elicited by electrical stimulation. However, intravenous administration of the beta-adrenergic blocking agent propranolol consistently abolished the increase in heart rate elicited by stimulation of five sites in the hypothalamus (Table 1).

TABLE 1. Changes in heart rate (bpm) and in mean arterial pressure (mmHg) elicited by electrical stimulation of sites in the posteromedial hypothalamus before and after vagotomy and administration of propranolol.

Preparation (n = number of sites stimulated)	Cardioacceleration		Arterial Hypertension	
	Before	After	Before	After
Intact (n=20)	+32 ± 3.5		+46 ± 7.4	
Vagotomy (n=3)	+28 ± 4.1	+27 ± 4.4*	+39 ± 13.5	+39 ± 12.3*
Propranolol (n=5)	+32 ± 7.9	+ 3 ± 2.2**	+47 ± 13.2	+28 ± 7.7*

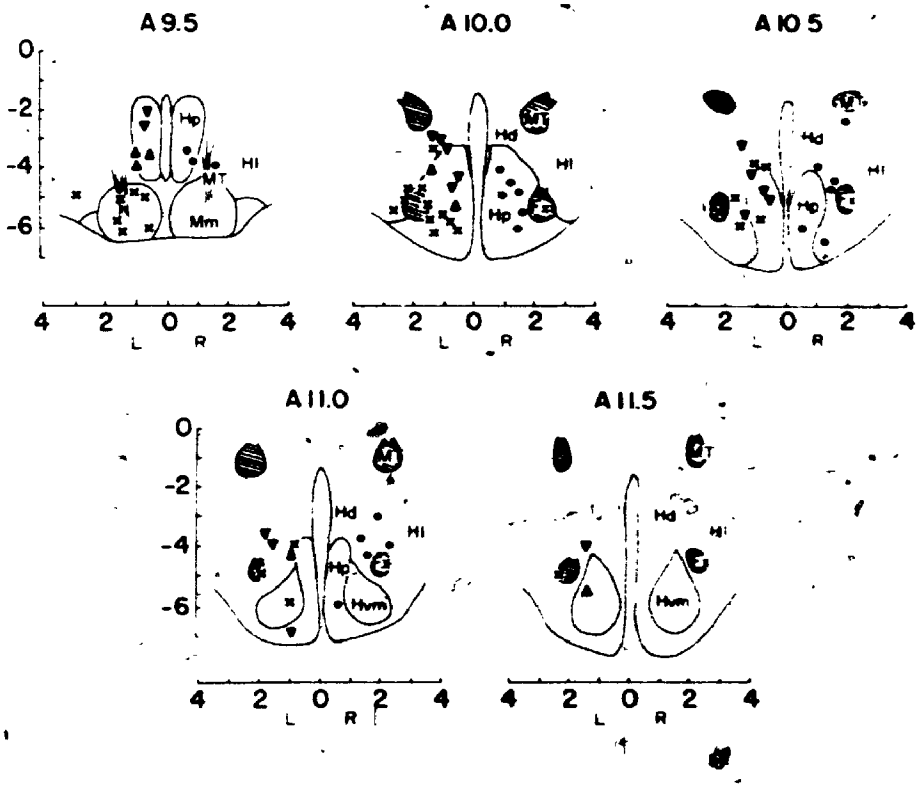
Values are means ± S.E.

* $p > 0.5$

** $p < 0.05$

Figure 3

Location of iron deposits corresponding to hypothalamic sites of stimulation and recording in 23 cats. The transverse sections of the hypothalamus are modified from a stereotaxic atlas (Jasper & Ajmone Marsan, 1954). Each section includes sites located between 0.25 mm cranial and 0.25 mm caudal to the plane of section. For convenience stimulation sites are shown on the right and recording sites on the left. ●, location of 20 sites at which electrical stimulation elicited cardioacceleration and arterial hypertension. ▲, location of 7 units that were excited by CSN stimulation. ▼, location of 16 units that were inhibited. ✖, location of 28 units that did not respond. Hd, dorsal hypothalamus; Hl, lateral hypothalamus; Hp, posterior hypothalamus; Hvm, ventromedial hypothalamus, Mm, mammillary bodies; Fx, fornix; MT, mammillo-thalamic tract.


A 9.5
A 10.0
A 10.5
A 11.0
A 11.5
0
-2
-4
-6
Hp
MT
Mm
Hl
Hd
Hvm
4 2 0 2 4
L R

B. Effect of baroreceptor and chemoreceptor excitation on activity of single units in the posteromedial hypothalamus

(a) Responses of units to electrical stimulation of the carotid sinus nerve

As it had been shown that electrical stimulation of a discrete region in the posteromedial hypothalamus produced marked increases in heart rate and arterial pressure, the effect of stimulation of the ipsilateral carotid sinus nerve (CSN) on the activity of single units in this region was investigated in 14 cats. Twenty-three of the 51 units from which recordings were obtained responded to stimulation of the CSN and 28 units did not respond; these latter units were generally located either ventral or lateral to the area where responsive units were found. The firing frequency of the 23 responsive units was altered by stimulation of the CSN: seven units were excited and 16 were inhibited. The mean spontaneous rates of discharge of the excited (5.7 ± 4.1, range 0 to 30 spikes/sec) and of the inhibited (5.7 ± 0.9, range 2.3 to 18.0 spikes/sec) neurones were not significantly different. Although the ranges of latencies of the two responses overlap, the mean latencies of the response of excited and inhibited neurones were significantly different ($p<0.05$). Furthermore, the mean duration of the inhibitory response was significantly greater ($p<0.001$) than the duration of the excitatory response. These results are summarized in Table 2 and the location of iron deposits corresponding to the sites of recording is shown in Figure 3. Typical records of an excitatory and of an inhibitory response to stimulation of the CSN are

TABLE 2. Response of single units in the posteromedial hypothalamus to electrical stimulation of the ipsilateral carotid sinus nerve.

Response	Latency (msec)	Duration (msec)
Neurones excited (n = 7)	29 ± 3.7 (range 17-40)	19 ± 4.9 (range 5-40)
Neurones inhibited (n = 16)	68 ± 9.0* (range 30-140)	263 ± 23.2** (range 90-420)

Values are means ± S.E.

*P<0.05

**P<0.001

seen in Figure 4.

(b) Response of units to selective stimulation of baroreceptors and chemoreceptors

As it is known that the CSN contains baroreceptor and chemoreceptor A-fibres (Fidone & Sato, 1969; Heymans & Neil, 1958) it is likely that both baroreceptor and chemoreceptor fibres were excited by the electrical stimulation of the CSN. To determine whether the response of single units in the posteromedial hypothalamus was due to stimulation of baroreceptor or chemoreceptor fibres in the CSN the response of some hypothalamic units to electrical stimulation of the CSN was compared with the response of the same units to selective stimulation of either baroreceptors or chemoreceptors. As it has been shown that the bradycardia induced by noradrenaline (NA) is due to baroreceptor excitation (Gebber & Snyder, 1970) and the bradycardia observed after close intra-arterial injection of sodium cyanide (NaCN) is due to stimulation of carotid body chemoreceptors (Daly & Scott, 1958; Jacobs, Sampson & Comroe, 1971; Scott, 1966), in this investigation the period of bradycardia observed after the injection of NA or NaCN was considered to be the time of selective activation of baroreceptors or chemoreceptors.

The effect of selective excitation of baroreceptors on hypothalamic unit activity was investigated in five units that were inhibited by CSN stimulation and it was found that the discharge frequency of these units was markedly decreased during the baroreceptor induced bradycardia after NA injection. The response to NA administration of a hypothalamic unit that was inhibited by CSN stimulation is shown in

Figure 4

Post-stimulus time histograms (upper) and photographic records (lower) of activity of single units affected by electrical stimulation of the carotid sinus nerve (CSN). A. from a single unit excited by CSN stimulation. B. from a single unit inhibited by CSN stimulation, lower trace is 3 super-imposed sweeps. N = number of responses summated for PST histograms. Stimulus occurred at time zero in each case.

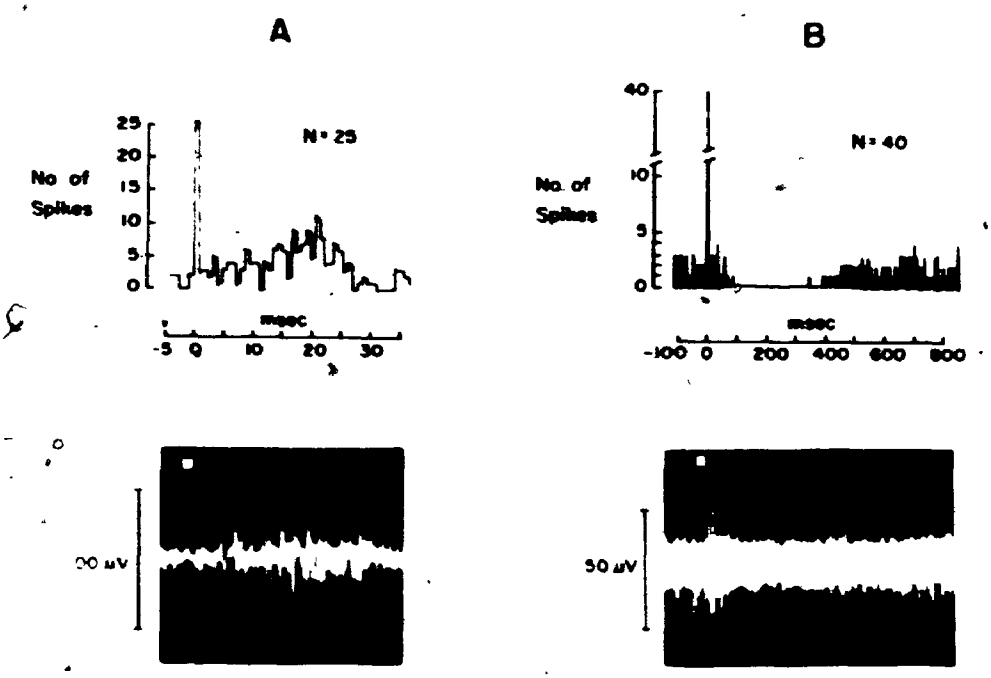

A
B
No of Spikes
N = 25
msec
-5 0 10 20 30
No. of Spikes
N = 40
msec
-100 0 200 400 600 800
50 μV
50 μV

Figure 5.

In addition, NaCN was administered while recording from three of these five units. Although NaCN injection elicited a marked chemoreceptor induced bradycardia, no significant change in the frequency of discharge was observed.

The effect of selective excitation of carotid body chemoreceptors on hypothalamic unit activity could be investigated in only one unit that was excited by CSN stimulation, and it was found that its discharge frequency was markedly increased during the chemoreceptor induced bradycardia (Fig. 6); however, administration of NA did not alter its firing rate.

Finally, in three units that did not respond to CSN stimulation no obvious change in firing frequency was induced by NA administration, and in two of these chemoreceptor activation also failed to influence the frequency of firing. These results are summarized in Table 3.

C. Cardiovascular responses elicited by stimulation of carotid body chemoreceptors

Intracarotid injection of NaCN in 29 animals consistently elicited bradycardia and arterial hypertension. The cardiovascular responses appeared within 5 sec after NaCN injection; a typical response is shown, in Figure 7A. The mean values of changes in heart rate and arterial pressure elicited by chemoreceptor stimulation are presented in Table 4. Although changes in respiratory frequency were usually observed to accompany the cardiovascular response (an increase in 26 animals and no change in 3) a tachycardia secondary to the polypnea

Figure 5

Response of a single unit inhibited by carotid sinus nerve (CSN) stimulation to selective excitation of baroreceptors. A, spontaneous discharge. B, discharge during noradrenaline (NA) induced reflex bradycardia (10 sec after A). In each record, top to bottom: nerve discharge, arterial pressure pulse, time marker (sec). Note the marked decrease in discharge frequency during baroreceptor induced bradycardia.

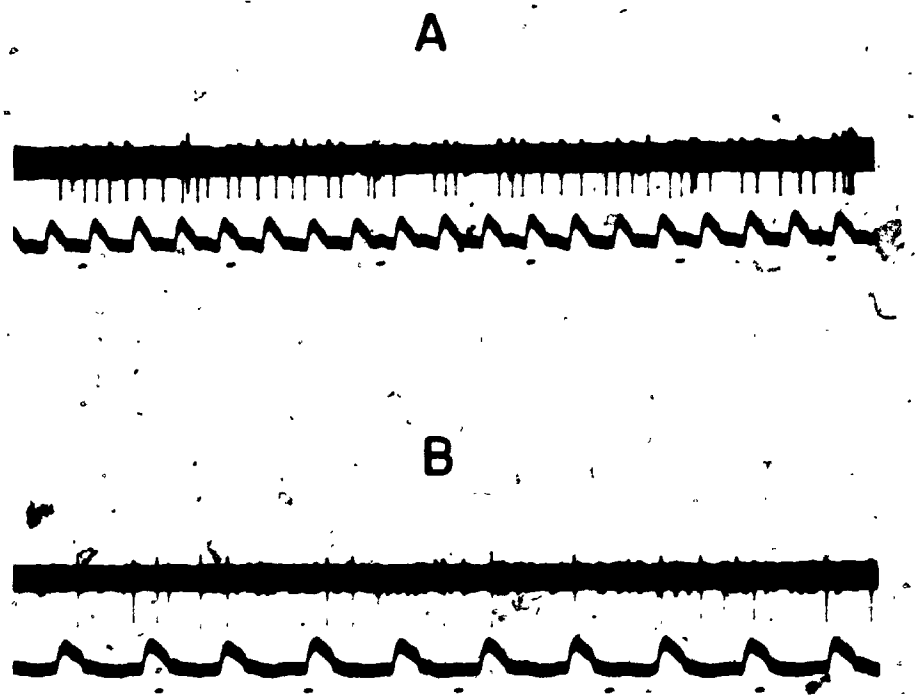

A
B
50 µV

Figure 6

Response of a single unit excited by carotid sinus nerve (CSN) stimulation to selective activation of carotid body chemoreceptors. A, spontaneous discharge. B, discharge during sodium cyanide (NaCN) induced reflex bradycardia (10 sec after A). In each record, top to bottom: nerve discharge, arterial pressure pulse, time marker (sec). Note the marked increase in discharge frequency during chemoreceptor induced bradycardia.

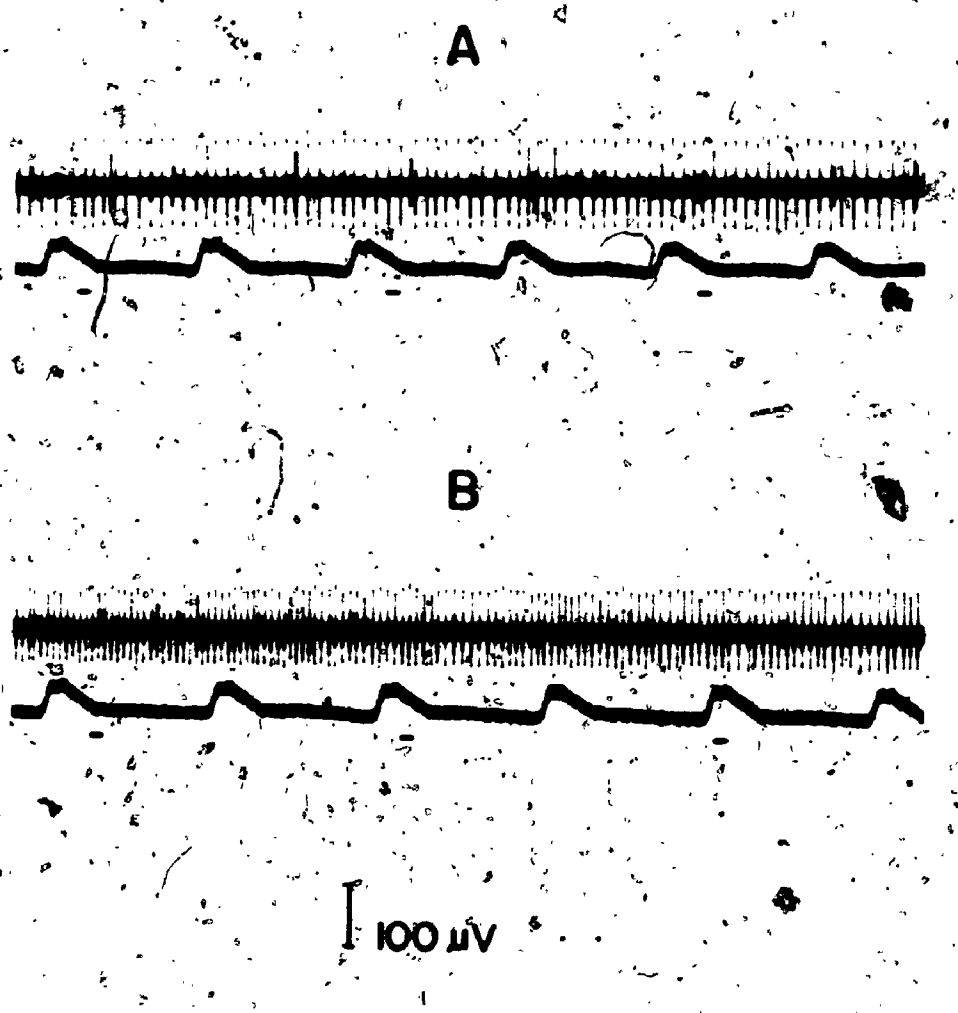

A
B
100 µV

TABLE 3. Responses of single units in the posteromedial hypothalamus to electrical stimulation of the carotid sinus nerve, to selective stimulation of baroreceptors and to selective stimulation of carotid body chemoreceptors.

Response to CSN stimulation	Response to NA injection		Response to NaCN injection	
	% change in discharge frequency	% change in heart rate	% change in discharge frequency	% change in heart rate
Inhibition	- 46	- 31		
Inhibition	- 28	- 62		
Inhibition	- 54	- 40	+ 2	- 22
Inhibition	- 87	- 50	- 7	- 26
Inhibition	- 45	- 30	- 2	- 18
No response	+ 4	- 21		
No response	- 12	- 30	- 2	- 25
No response	- 7	- 38	- 3	- 29
Excited	- 3	- 41	+140	- 37

All values are means of three runs rounded to the nearest integer.

Figure 7

Effect of administration of propranolol and of vagotomy on cardiovascular response to excitation of chemoreceptors. In each record from top to bottom: arterial pressure, tachograph tracing, respiration; at the arrow intracarotid injection of sodium cyanide (NaCN) 50 ug. A, control response and B, response after 1.5 mg propranolol I.V. C, response after right vagotomy and D, after bilateral vagotomy.

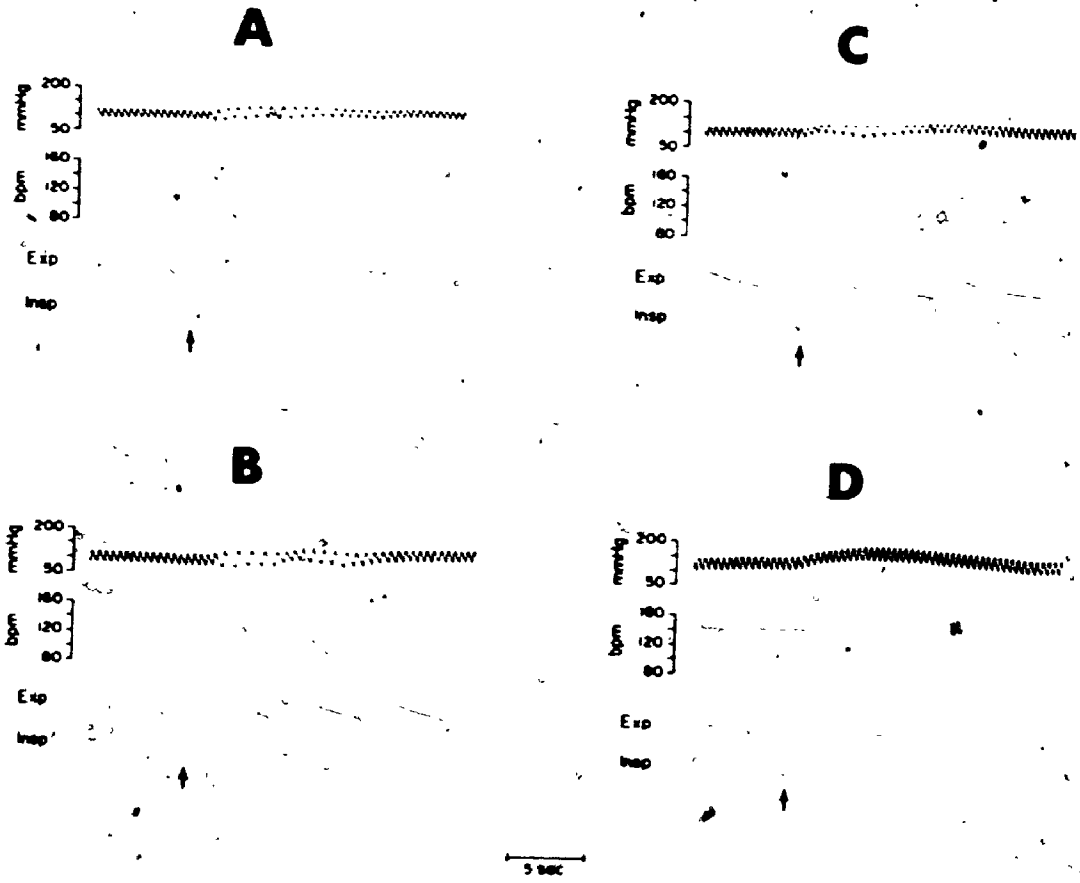

A
B
C
D
mmHg
200
50
bpm
160
120
80
Exp
Insp
5 sec

TABLE 4. Effect of administration of propranolol and of vagotomy on cardiovascular responses elicited by injection of sodium cyanide into right carotid artery.

	Change in heart rate (bpm)		Change in mean arterial pressure (mmHg)	
Preparation	Before	After	Before	After
Control (n = 29)	-28 ± 6.8		+25 ± 5.7	
Propranolol (n = 9)	-24 ± 2.8	-20 ± 2.3	+21 ± 3.7	+23 ± 4.8
Right vagotomy (n = 5)	-30 ± 6.2	-19 ± 5.1*	+22 ± 4.0	+21 ± 4.8
Bilateral vagotomy (n = 5)	-30 ± 6.2	+ 2 ± 2.1*	+22 ± 4.0	+26 ± 5.5

Values are means ± S.E.
n = number of animals
Resting heart rate: control, 197 ± 9.2; propranolol, 163 ± 4.2; right vagotomy 187 ± 16.6; bilateral vagotomy, 187 ± 16.6.

*P<0.05 for difference between responses elicited before and after experimental procedure.

was never observed. In addition, in two experiments NaCN induced bradycardia and arterial hypertension were found to be abolished by section of the carotid sinus nerve.

(a) Effect of administration of propranolol and of bilateral cervical vagotomy on chemoreceptor induced bradycardia

To investigate the efferent mechanism mediating the chemoreceptor bradycardia the cardiovascular responses elicited by carotid body chemoreceptor excitation before and after blocking either the sympathetic or parasympathetic input to the heart were compared. In 9 cats administration of propranolol did not significantly alter the cardiac response to intracarotid injection of NaCN ($p>0.3$). However, in another group of 5 animals the cardiac slowing was significantly ($p<0.05$) reduced by right vagotomy and abolished by bilateral cervical vagotomy ($p<0.05$). These results are summarized in Table - and typical responses to chemoreceptor stimulation before and after either administration of propranolol or vagotomy are shown in Figure 7.

Although these results indicated that the chemoreceptor induced bradycardia was not due to inhibition of the sympathetic input to the heart, the possibility existed that the reflex vagal bradycardia was not due solely to carotid body chemoreceptor excitation but also to vagal discharge induced reflexly by activation of baroreceptors during the arterial hypertension elicited by chemoreceptor stimulation. To investigate this possibility, experiments were done to compare the magnitude of the cardiac slowing elicited by intracarotid injection of NaCN before and after eliminating the pressor response by spinal tran-

section.

(b) Effect of spinal transection and bilateral cervical vagotomy on chemoreceptor bradycardia

In 10 cats the magnitude of cardiac slowing elicited by intracarotid injection of NaCN was not affected by spinal transection at C_7. In addition, in two animals that required artificial ventilation after spinal transection, the chemoreceptor bradycardia was similar in magnitude to that obtained in spontaneously breathing cats. A typical response before and after transection is shown in Figure 8. In 7 of these cats the effect of vagotomy on the chemoreceptor bradycardia was investigated and it was shown that the bradycardia was significantly ($p<0.05$) reduced by section of the right cervical vagus and abolished by bilateral vagotomy ($p<0.05$). These results are summarized in Table 5. It is worth noting by inspection of Figure 8 and Table 5 that in spinal animals the bradycardia elicited by intracarotid injection of NaCN was accompanied by a slight decrease in arterial pressure probably related to the decreased cardiac output which is observed during cardiac slowing.

(c) Effect of midcollicular decerebration on chemoreceptor bradycardia

As electrical stimulation of the posteromedial hypothalamus (PMH) elicited pressor responses similar to the response elicited by

Figure 8

Effect of spinal transection at C_7, on cardiovascular response to excitation of chemoreceptors. In each record from top to bottom: arterial pressure, tachograph tracing, respiration; at the arrow intracarotid injection of sodium cyanide (NaCN) 60 ug. A, control response. B, after spinal transection.

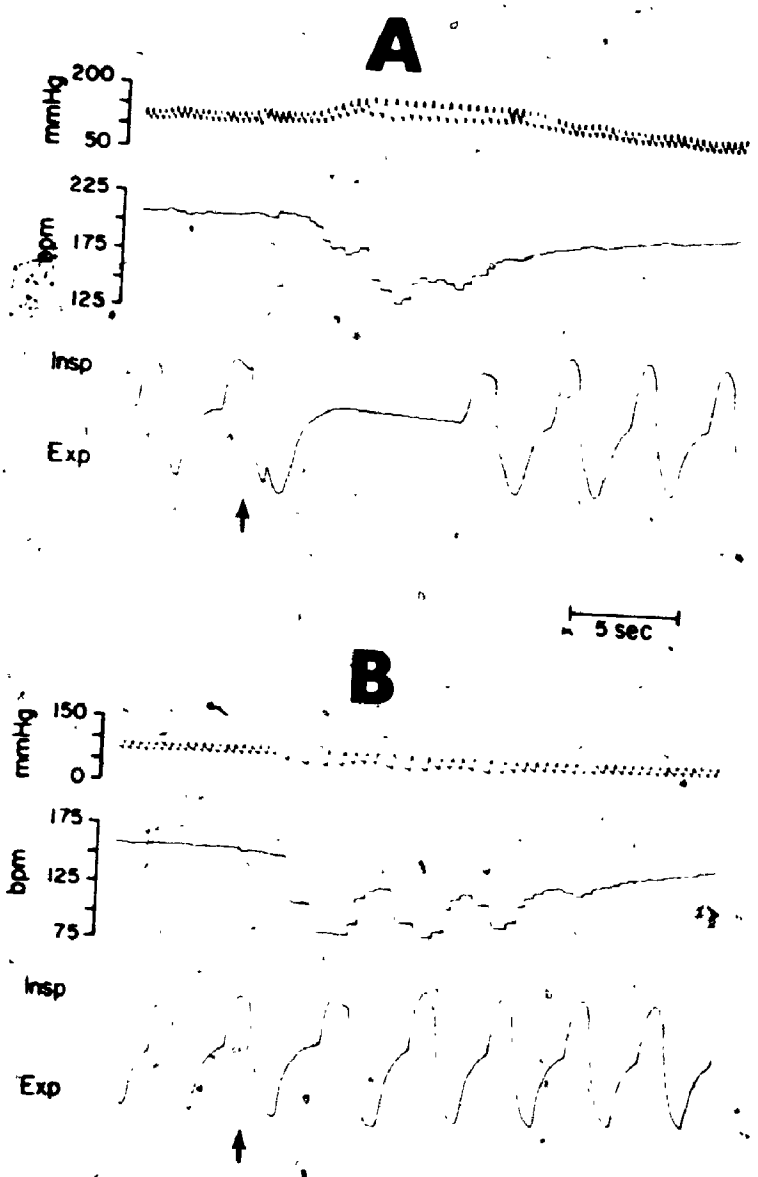

A
mmHg
200
50
225
bpm
175
125
Insp
Exp
5 sec
B
mmHg
150
0
175
bpm
125
75
Insp
Exp

TABLE 5. Effect of spinal transection and of vagotomy on cardiovascular responses elicited by injection of sodium cyanide into right carotid artery.

Preparation	Change in heart rate (bpm)		Change in mean arterial pressure (mmHg)	
	Before	After	Before	After
Spinal transection (n = 10)	-[illegible] ± 9.6	-[illegible] ± 7.5	+[illegible] ± [illegible]	-[illegible] ± 2.9*
Right vagotomy (n = 7)	-[illegible] ± [illegible]	-[illegible] ± [illegible]*	-[illegible] ± [illegible]	-[illegible] ± [illegible]
Bilateral vagotomy (n = 7)	-[illegible] ± [illegible]	0*	-[illegible] ± 2.9	0*

Values are mean ± S.E.

n = number of animals

*P < 0.05 for difference between responses elicited before and after experimental procedure

chemoreceptor excitation and chemoreceptors had been shown to project to the PMH, the possibility that the PMH was involved in the mediation of the cardiovascular response elicited by chemoreceptor excitation was investigated by comparing the magnitude of the cardiovascular changes elicited by stimulation of chemoreceptors before and after midcollicular decerebration. Midcollicular decerebration was done in four chloralosed animals; a significant ($p<0.05$) increase in the magnitude of the cardiac slowing compared to the response elicited in the intact preparation was observed. In addition, the chemoreceptor bradycardia elicited after decerebration was significantly ($p<0.05$) reduced by right cervical vagotomy and abolished by bilateral vagotomy ($p<0.05$), results similar to those obtained in animals with an intact central nervous system and in spinal animals. These experiments are summarized in Table 6 and typical responses to chemoreceptor excitation before and after decerebration and the effect of vagotomy on the bradycardia are shown in Figure 9.

D. Effect of electrical stimulation of the posteromedial hypothalamus on baroreceptor and chemoreceptor induced bradycardia in spinal animals

The finding that midcollicular decerebration potentiated chemoreceptor bradycardia (Table 6; Fig. 9) suggested the possibility that suprabulbar structures may inhibit the chemoreceptor reflex. As chemoreceptor excitation alters the discharge frequency of single units in the posteromedial hypothalamus (Table 3; Fig. 6), the effect of hypothalamic stimulation on the bradycardia induced by carotid sinus

TABLE 6. Effect of midcollicular decerebration and of vagotomy on cardiovascular responses elicited by injection of sodium cyanide into right carotid artery.

	Change in heart rate (bpm)		Change in mean arterial pressure (mmHg)	
Preparation	Before	After	Before	After
Decerebration (n = 4)	-31 ± 4.0	-71 ± 8.5*	+18 ± 2.0	+19 ± 5.2
Right vagotomy (n = 4)	-71 ± 8.5	-36 ± 2.0*	+19 ± 5.2	+19 ± 3.5
Bilateral vagotomy (n = 4)	-7[illegible] ± [illegible]	0*	+19 ± 5.[illegible]	+39 ± 7.0

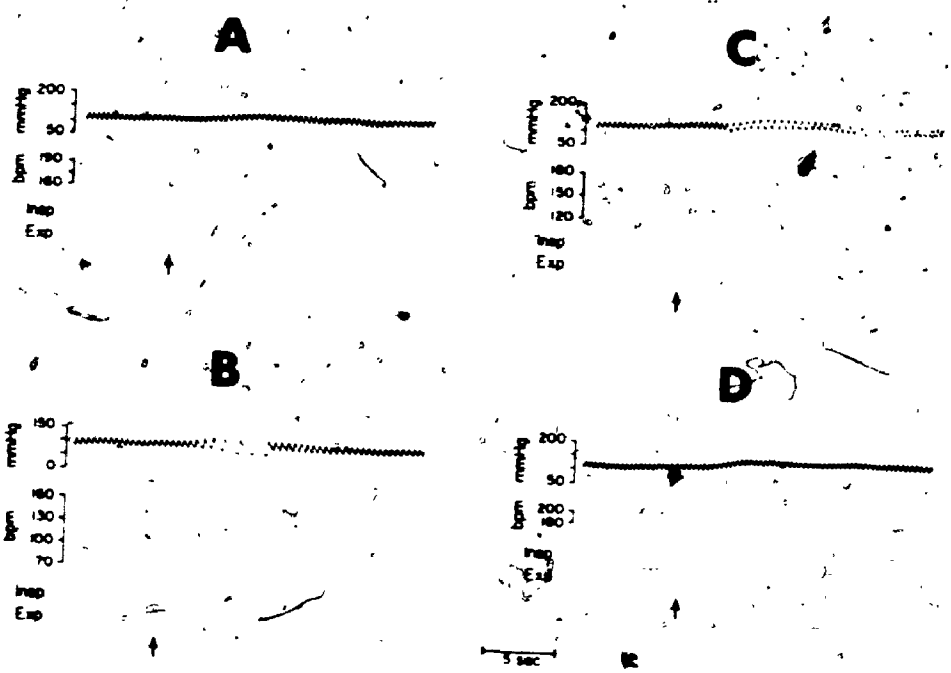
Values are mean ± S.E.

n = number of animals

*P<0.05 for difference between responses before and after experimental procedure

Figure 9

Effect of midcollicular decerebration and of vagotomy on cardiovascular response to excitation of chemoreceptors. In each record from top to bottom: arterial pressure, tachograph tracing, respiration; at the arrow intracarotid injection of sodium cyanide (NaCN) 80 ug. A, control response and B, response after decerebration. C, after right vagotomy. D, after bilateral vagotomy.


A
mmHg
200
50
bpm
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bpm
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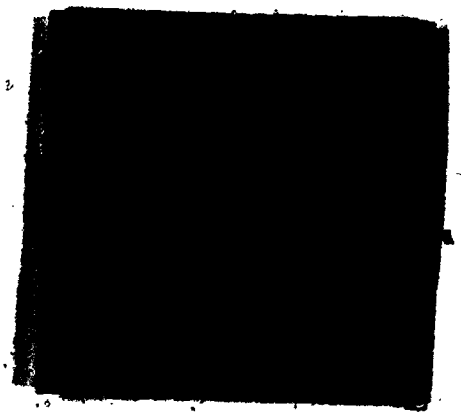

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nerve stimulation was investigated in order to ascertain whether or not the posteromedial hypothalamus could inhibit reflex vagal bradycardia.

Electrical stimulation of the right CSN in 8 animals transected at C_7 elicited a mean decrease in heart rate of 31 ± 1.9 beats/min. Selective electrical stimulation of 28 ipsilateral and 13 contralateral sites in the posteromedial hypothalamus, which in the intact animal elicits cardioacceleration and arterial hypertension (Table 1) did not elicit any changes in heart rate or arterial pressure in these spinal animals. However, when both the CSN and these sites in the hypothalamus were stimulated simultaneously the decrease in heart rate elicited by CSN stimulation was reduced significantly ($p<0.001$) as shown in Table 7. The location of iron deposits corresponding to the sites of stimulation is shown in Figure 10. A typical response in which the bradycardia elicited by stimulation of the CSN was inhibited by simultaneous stimulation of the hypothalamus is presented in Figure 11.

After the inhibitory effect of hypothalamic stimulation on the reflex bradycardia elicited by CSN stimulation was demonstrated, to elucidate whether the inhibition was affecting the chemoreceptor or baroreceptor input, the posteromedial hypothalamus was stimulated during selective activation of either chemoreceptors or baroreceptors. Intracarotid injection of 50 - 100 µg of NaCN in 10 cats transected at C_7 consistently elicited cardiac slowing (Fig. 12C). Electrical stimulation of 22 ipsilateral and 17 contralateral sites in the posteromedial hypothalamus failed to alter heart rate in these animals but did

TABLE 7. Effect of hypothalamic stimulation on the reflex vagal bradycardia elicited either by electrical stimulation of the carotid sinus nerve (CSN) (8 cats) or by selective excitation of carotid body chemoreceptors and baroreceptors (10 cats).

Preparation	Magnitude of Bradycardia (bpm)	% Inhibition
CSN	31 ± 1.4	
CSN + R. Hypothalamus (n = 28)	10 ± 1.2*	68%
CSN	30 ± 2.4	
CSN + L. Hypothalamus (n = 13)	8 ± 1.9*	73%
NaCN	33 ± 2.0	
NaCN + R. Hypothalamus (n = 22)	9 ± 1.6*	73%
NaCN	39 ± 6	
NaCN + L. Hypothalamus (n = 17)	13 ± 3*	67%
NA	44 ± 1.9	
NA + R. or L. Hypothalamus (n = 39)	8 ± 2.6*	82%

Values are mean ± S.E.

n = number of experimental runs

*P<0.001 for changes during simultaneous stimulation compared with changes elicited by CSN or chemoreceptor or baroreceptor stimulation alone.

Figure 10

Location of electrode tips corresponding to sites of stimulation in the hypothalamus in 18 cats. Each section includes sites located between 0.25 mm cranial and 0.25 mm caudal to the plane of section. ●, location of 41 sites at which stimulation inhibited the vagal bradycardia elicited by CSN stimulation; ✖, location of 39 sites that inhibited both baroreceptor and chemoreceptor induced vagal bradycardia; Fx, fornix; Hd, dorsal hypothalamus; Hl, lateral hypothalamus; Hp, posterior hypothalamus; Hvm, ventromedial hypothalamus; Mm, mammillary bodies; MT, mammillothalamic tract.

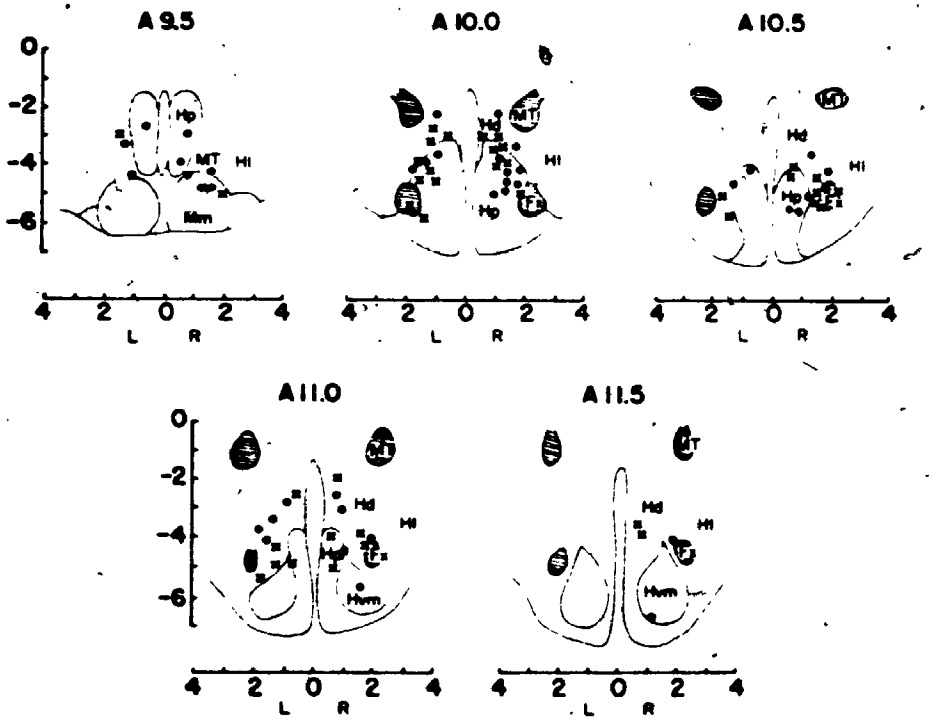

A 9.5
A 10.0
A 10.5
A 11.0
A 11.5
0
-2
-4
-6
Hp
MT
HI
Mm
Hd
Hvm
4 2 0 2 4
L R

Figure II

Inhibition of carotid sinus nerve (CSN) induced vagal bradycardia by electrical stimulation of the postero-medial hypothalamus. A, stimulation of CSN. B, stimulation of hypothalamus. C, simultaneous stimulation of CSN and of hypothalamus. In each record from top to bottom: arterial pressure, tachograph tracing, electrical stimulus.

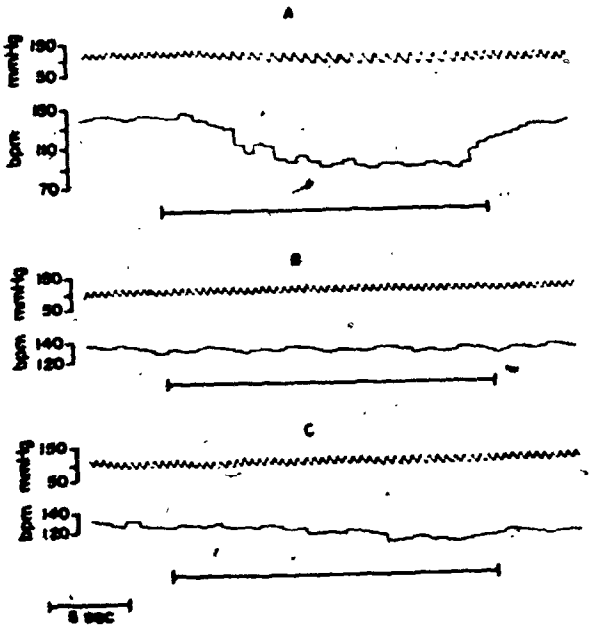
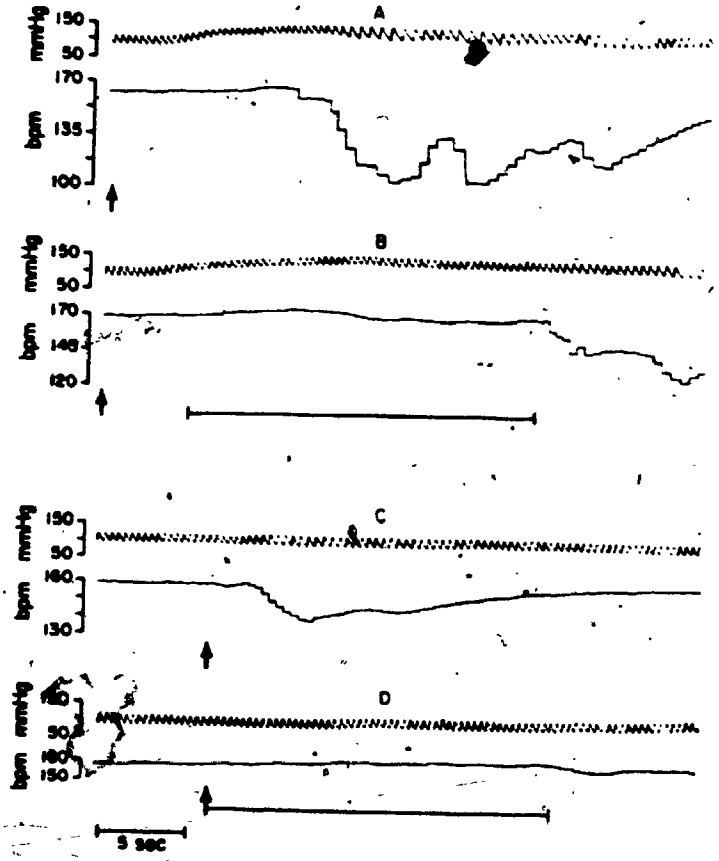

A
mmHg
150
50
bpm
150
110
70
B
mmHg
150
50
bpm
140
120
C
mmHg
150
50
bpm
140
120
5 sec

Figure 12

Inhibition of chemoreceptor and baroreceptor induced vagal bradycardia by electrical stimulation of the posteromedial hypothalamus. A, selective excitation of baroreceptors (noradrenaline (NA), 1.0 ug/kg I.V.). B, simultaneous stimulation of baroreceptors and of posteromedial hypothalamus. C, selective excitation of right carotid body chemoreceptors (sodium cyanide, (NaCN) 50 ug.). D, simultaneous stimulation of chemoreceptors and of posteromedial hypothalamus. In each record from top to bottom: arterial pressure, tachograph tracing, electrical stimulus. Injections of NA and NaCN were given at the arrow.


A
mm Hg
150
50
bpm
170
135
100
B
mm Hg
150
50
bpm
170
140
120
C
mm Hg
150
50
bpm
160
130
D
mm Hg
150
50
bpm
160
150
5 sec

inhibit significantly ($p<0.001$) the cardiac slowing elicited by chemoreceptor excitation (Fig. 12D). In addition, electrical stimulation of the same 39 hypothalamic sites that inhibited chemoreceptor bradycardia also inhibited significantly ($p<0.001$) the baroreceptor bradycardia (Fig. 12B) elicited by intravenous administration of 0.5 - 2.0 µg/kg of noradrenaline (Fig. 12A). The mean values of these changes in heart rate are presented in Table 7 and the location of iron deposits corresponding to the sites of stimulation is shown in Figure 10.

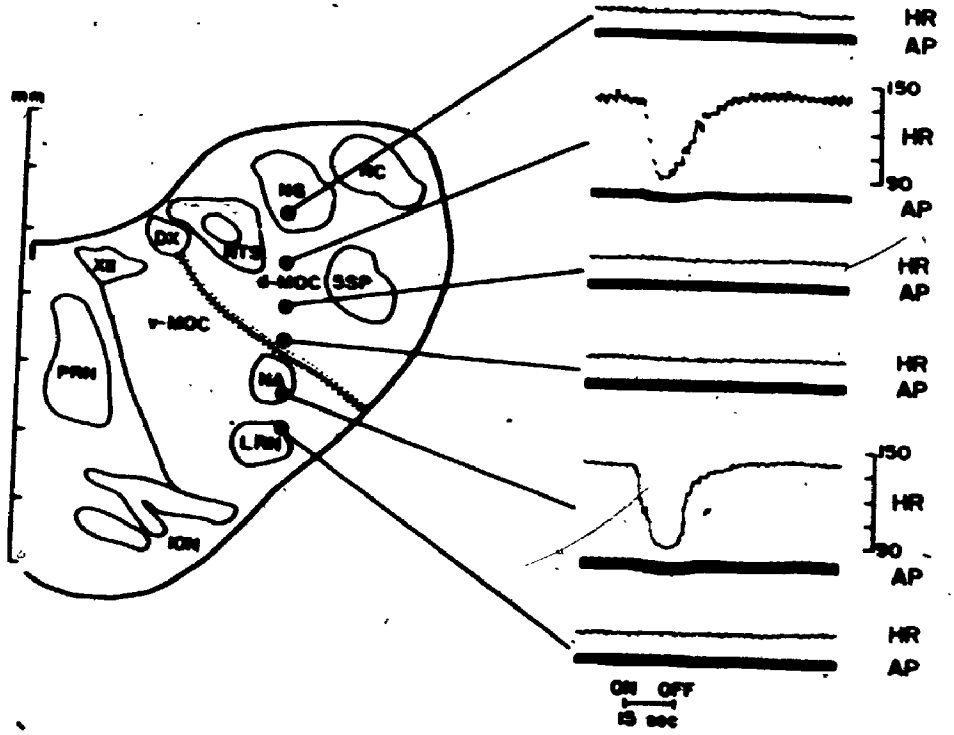
The experiments reported in this section clearly demonstrated that the posteromedial hypothalamus (PMH) had an inhibitory effect on the vagal bradycardia elicited by activation of baroreceptors and chemoreceptors. To investigate the mechanism of this inhibitory interaction, attempts were made to record field potentials in the medulla during electrical stimulation of the PMH. As the medullary structures involved in the mediation of reflex vagal bradycardia are uncertain, it was considered a necessary prerequisite to identify these medullary structures before attempting to locate the sites at which the hypothalamus inhibited the vagal bradycardia elicited by baroreceptor and chemoreceptor stimulation.

E. Localization of medullary cardioinhibitory sites in spinal cats

The right side of the medulla in 27 cats transected at C_7 was explored systematically in a region extending from 1 mm caudal to 4 mm rostral to the obex and from the midline to 5 mm lateral. The typical cardiovascular responses elicited by electrical stimulation of sites in this region during an electrode penetration are shown in Figure 13; vagal bradycardia appeared within 1 - 2 sec after onset of

Figure 13

Cardiovascular responses elicited by electrical stimulation of the medulla at different depths of penetration. Note a dorsal and a ventral cardio-inhibitory site separated by an unresponsive region. NA, n. ambiguus; NTS, n. of tractus solitarius; d -, v - MOC, dorsal, ventral n. medullae oblongatae centralis; PRN, paramedian reticular n; LRN, lateral reticular n.; 5SP, n. tractus spinalis trigemini; NG, n. gracilis; NC, n. cuneatus; ION, inferior olivary n.; XII, n. hypoglossi; DX, dorsal n. of vagus.


mm
NTS
NC
DX
XII
d-MOC
SSP
v-MOC
PRN
NA
L.RN
IORN
HR
AP
150
HR
90
AP
HR
AP
HR
AP
150
HR
90
AP
HR
AP
ON OFF
15 sec

stimulation and was elicited by stimulation at two sites, one in the dorsal and one in the ventral medulla, which were separated by an unresponsive region. The location of iron deposits corresponding to 160 cardioinhibitory sites is shown in Figure 14. As most of the sites appeared to be located in two separate dorsal and ventral regions they were arbitrarily classified as either dorsal or ventral with respect to the intramedullary rootlets of the vagus. However, some of the sites located in a medial region could not be readily classified as either dorsal or ventral to the vagal rootlets and were therefore classified as dorsomedial. The distribution of dorsal, ventral and dorsomedial cardioinhibitory sites in medullary structures is summarized in Table 8, which shows that the majority of the dorsal sites (41/51) were located in the nucleus of tractus solitarius (NTS) (23) and dorsal nucleus medullae oblongatae centralis (MOC) (18), that the majority of the ventral sites (86/97) were located in the nucleus ambiguus (NA) (55) and ventral MOC (31) and that the dorsomedial sites (12) were located in a region ventral to the dorsal nucleus of the vagus and between the ventral MOC and the hypoglossal nucleus. The mean values of changes in heart rate and arterial pressure elicited by stimulation of the three different locations (dorsal, ventral and dorsomedial sites) were not significantly different from one another; these results are summarized in Table 9.

In 21 animals breathing spontaneously, changes in respiratory frequency were usually observed to accompany the cardiac slowing: a decrease in respiratory frequency was noted at 106 sites, an increase at 5 and no change at 7. In addition, in six animals that required artificial respiration after spinal transection bradycardia could still

Figure 14

Location of 160 iron deposits corresponding to sites of stimulation that elicited vagal bradycardia in 27 cats. The transverse sections of the medulla are modified from a stereotaxic atlas (Berman, 1968). The number in each section represents distance (mm) rostral (+) or caudal (-) to the obex. For identification of structures see Figure 13.

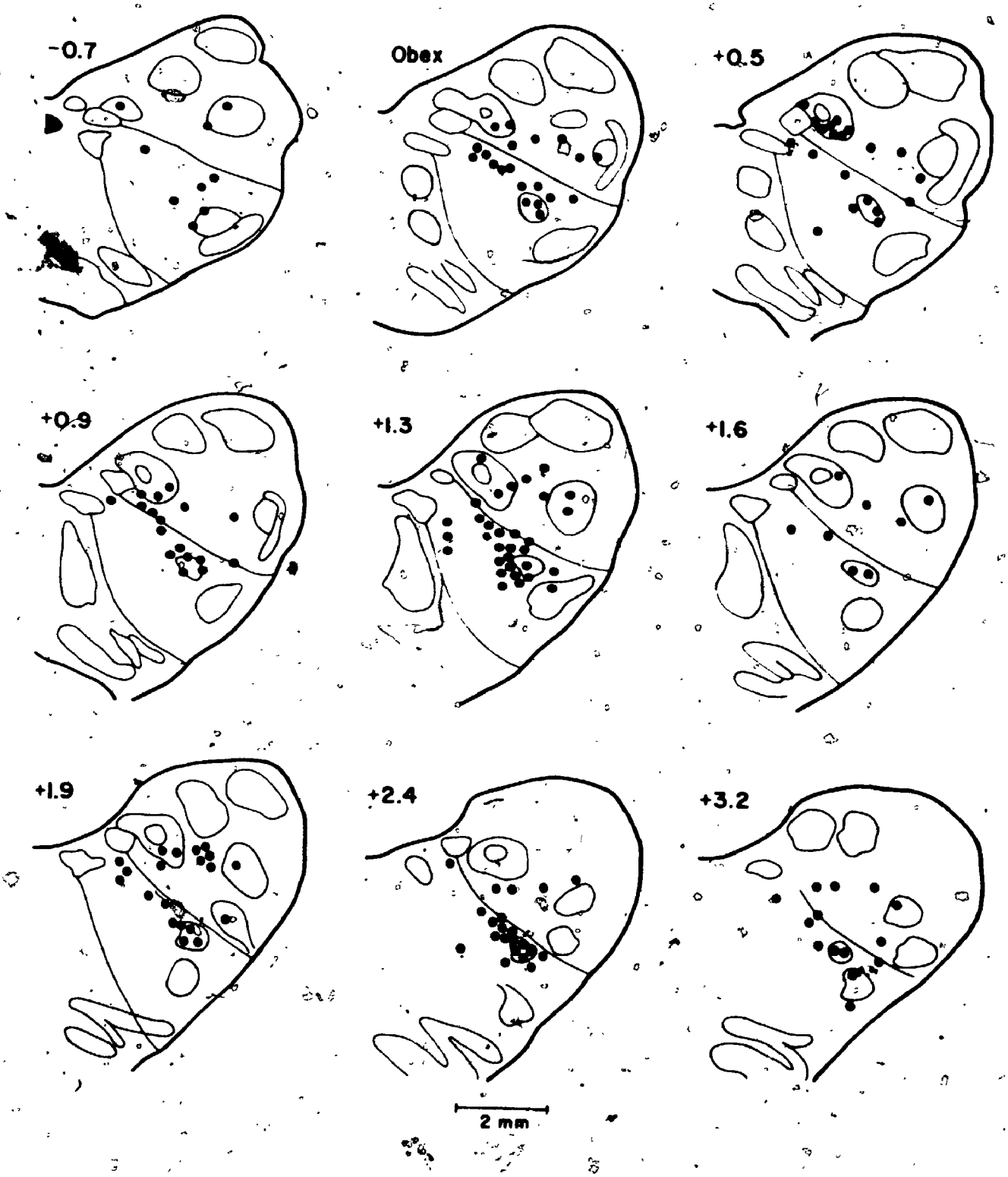

-0.7
Obex
+0.5
+0.9
+1.3
+1.6
+1.9
+2.4
+3.2
2 mm

TABLE 8. Distribution of 160 vagal cardioinhibitory sites in the medulla.

Dorsal (n=51)	nucleus tractus solitarius	23
	dorsal n. medullae oblongatae centralis	18
	n. tractus spinalis trigemini	10
Ventral (n=97)	nucleus ambiguus	55
	ventral n. medullae oblongatae centralis	31
	roctlets of vagus nerve	6
	lateral reticular nucleus	5
Dorsomedial (n = 12)	between n. hypoglossi and ventral MOC	12

TABLE 9. Changes in heart rate (bpm) and mean arterial pressure (mmHg) elicited by electrical stimulation of dorsal, ventral and dorsomedial sites in the right medulla.

	HR before stimulation	Change in HR during stimulation	Mean AP before stimulation	Change in mean AP during stimulation
Dorsal (n=51)	160 ± 4.2	-52 ± 4.4*	93 ± 3.0	-15 ± 1.3*
Ventral (n=97)	158 ± 4.6	-49 ± 2.7*	93 ± 2.3	-13 ± 0.9*
Dorsomedial (n = 12)	168 ± 10.2	-43 ± 6.9*	98 ± 5.4	-11 ± 2.1*

Values are means ± S.E.

n = number of sites stimulated

*$P<0.05$ for mean change in relation to zero effect.

be elicited by electrical stimulation (42 sites).

(a) Effect of ipsilateral and bilateral vagotomy on the bradycardia elicited by medullary stimulation

To determine whether the bradycardia elicited from stimulation of the right medulla was mediated by crossed or uncrossed pathways, the vagus nerve was cut first on the ipsilateral side and then on the contralateral side in 18 cats. After ipsilateral vagotomy electrical stimulation of 4 dorsal sites in the NTS and 14 ventral sites (11 in the NA and 3 in the ventral MOC) elicited a cardioacceleration which was abolished by section of the left vagus. These results are summarized in Table 10 and typical heart rate responses before and after vagotomy are shown in Figure 15.

(b) Effect of administration of sodium pentobarbital on the bradycardia elicited by medullary stimulation

Although the magnitudes of the bradycardia elicited from dorsal, ventral and dorsomedial sites were not significantly different and the effect of vagal section on the cardioinhibitory response from dorsal and ventral sites was similar, the results described in the preceding section did not exclude the possibility that the bradycardia observed was due to activation of different components of the same reflex arc. To test this hypothesis sodium pentobarbital (Nembutal Sodium, Abbott Laboratories, Montreal: 10-40 mg/kg i.v.), an agent which has been shown to depress synaptic transmission of baroreceptor and chemoreceptor inputs to the medulla (Miura & Reis, 1969) was administered to six

TABLE 10. Effect of vagotomy on the cardiac slowing elicited by stimulation of the right medulla.

Site of stimulation	Changes in Heart rate		
	Control	After ipsilateral vagotomy	After bilateral vagotomy
NTS (n=4)	-57 ± 5.1	+8 ± 2.4*	0*
v - MOC (n=3)	-39 ± 7.8	+8 ± 2.5*	0*
NA (n=11)	-50 ± 5.2	+16 ± 4.6*	0*

Values are mean ± S.E. in beats/min.

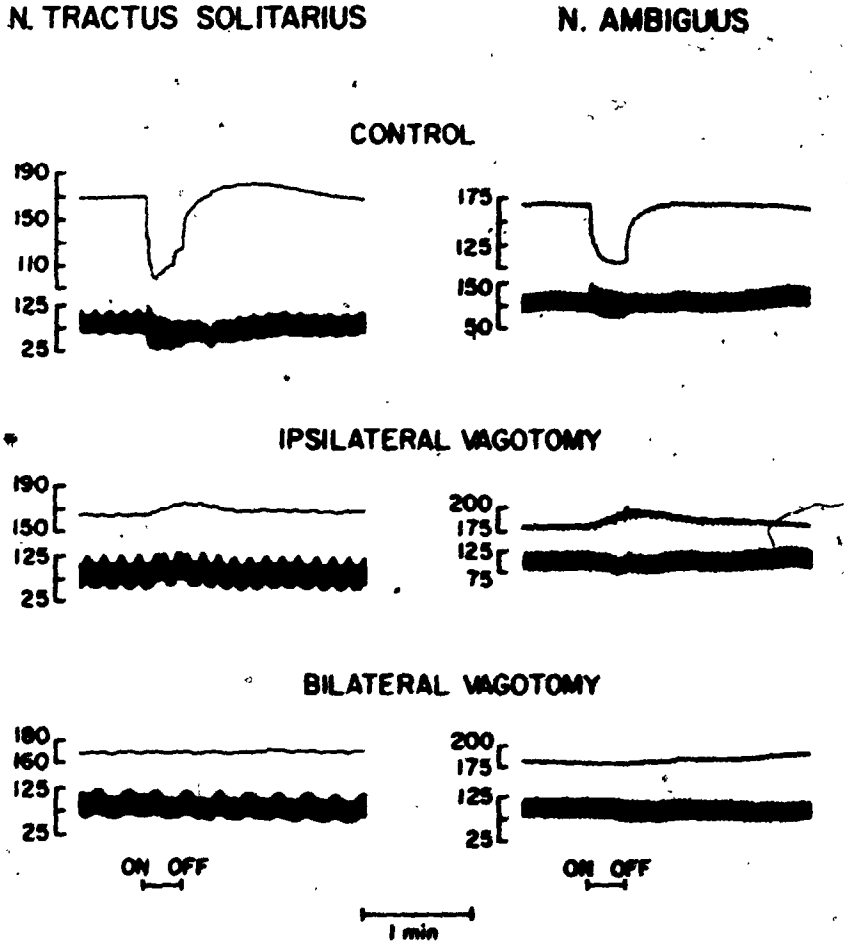
n = number of animals

*$P<0.05$ for difference between responses elicited before and after experimental procedure.

NTS, n. tractus solitarius; v-MOC, ventral n. medullae oblongatae centralis; NA, n. ambiguus.

Figure 15

Effect of ipsilateral and bilateral vagotomy on the bradycardia elicited by stimulation of the right medulla at sites in the n. ambiguus (NA) and in the n. of tractus solitarius (NTS). Note reversal of cardiac slowing to cardioacceleration after ipsilateral vagotomy. In each record: top, heart rate (beats/min); bottom, arterial pressure (mm Hg).


N. TRACTUS SOLITARIUS
N. AMBIGUUS
CONTROL
190
150
110
125
25
175
125
150
50
IPSILATERAL VAGOTOMY
190
150
125
25
200
175
125
75
BILATERAL VAGOTOMY
180
160
125
25
200
175
125
25
ON OFF
ON OFF
1 min

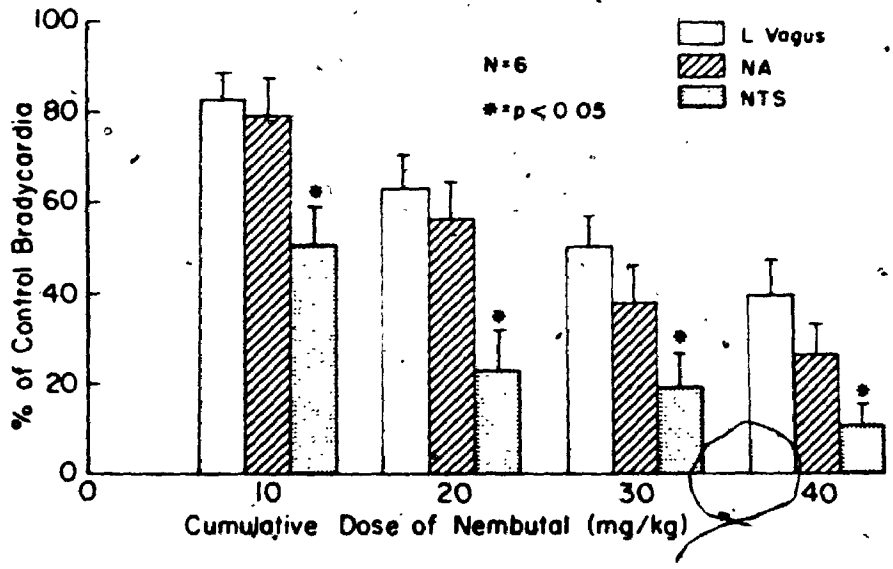
chloralosed cats and the magnitude of the bradycardia was compared to the control response. In addition, to eliminate the possibility that changes in the magnitude of the vagal bradycardia elicited by medullary stimulation might be due to a peripheral effect of sodium pentobarbital, the cardiac slowing elicited by stimulation of dorsal and ventral sites in the right medulla was compared to the bradycardia elicited by stimulation of the peripheral end of the cut left vagus in the same animal after administration of different doses of sodium pentobarbital. Administration of cumulative doses of sodium pentobarbital was found to decrease the magnitude of the vagal bradycardia elicited by stimulation of the peripheral end of the left cut vagus, the right NTS (dorsal sites) and the right NA region (ventral sites). The decreases in the magnitude of the bradycardia elicited by vagal stimulation and NA stimulation were not significantly different from each other even after injection of a cumulative dose of 40 mg/kg of sodium pentobarbital. However, injection of only 10 mg/kg of sodium pentobarbital significantly reduced the cardiac slowing elicited from the NTS with respect to the vagal and NA bradycardia, and injection of 40 mg/kg of sodium pentobarbital almost completely eliminated the response. These results are summarized in Figure 16.

F. Effect of electrical stimulation of the posteromedial hypothalamus on medullary bradycardia

To investigate the possible medullary sites at which the posteromedial hypothalamus (PMH) inhibits the vagal component of baroreceptor and chemoreceptor reflexes the effect of PMH stimulation

Figure 16

Heart rate responses (as a percent of control) elicited by stimulation of the peripheral segment of the cut left vagus (open bars), the right n. ambiguus (hatched bars), and the right n. of tractus solitarius (stippled bars) after cumulative doses of sodium pentobarbital administered i.v. to six chloralosed cats.


100
80
60
40
20
0
% of Control Bradycardia
0
10
20
30
40
Cumulative Dose of Nembutal (mg/kg)
N=6
*=p<0 05
L Vagus
NA
NTS

on the magnitude of the vagal bradycardia produced by stimulation of localized medullary regions that mediate vagal bradycardia (Table 8; Fig. 14) was studied in nine animals. Electrical stimulation of 10 sites in the right PMH and six sites in the left PMH that inhibited the reflex vagal bradycardia induced by intravenous administration of noradrenaline was found to inhibit the vagal bradycardia elicited by stimulation of 12 sites in the right medulla, seven in the nucleus tractus solitarius (NTS) and five in the dorsal part of the n. medullae oblongatae centralis (d-MOC). However, the bradycardia elicited by stimulation of 11 sites in the right nucleus ambiguus (NA) was not affected by PMH stimulation. These results are summarized in Table 11 and the location of sites of stimulation in the hypothalamus and in the medulla is shown in Figures 17 and 18. The characteristic cardiovascular responses are presented in Figure 19: the inhibition of NTS bradycardia is shown in Figure 19 A, B and the absence of inhibition during simultaneous stimulation of the PMH and NA is shown in Figure 19 C, D.

G. Field potentials evoked in the medulla during electrical stimulation of the posteromedial hypothalamus

As electrical stimulation of the PMH was found to inhibit the vagal bradycardia elicited by stimulation of well identified medullary nuclei; to investigate the possibility that these structures might be the site of termination of a descending hypothalamic inhibitory pathway attempts were made to record field potentials in the medulla during electrical stimulation of the PMH in 21 animals. Only sites of

TABLE 11. Effect of electrical stimulation of the ipsilateral (I) and contralateral (C) posteromedial hypothalamus (PMH) on the vagal bradycardia elicited by stimulation of the right medulla.

		DECREASE IN HEART RATE (bpm)	
		STIMULATION OF MEDULLA	STIMULATION OF MEDULLA AND PMH
n. tractus solitarius	(I) n = 5	50 ± 5.10	19 ± 4.76*
	(C) n = 2	46 ± 3.57	14 ± 3.29*
dorsal n. medullae	(I) n = 3	43 ± 2.19	18 ± 3.68*
obl. centralis	(C) n = 2	45 ± 4.81	21 ± 2.65*
n. ambiguus	(I) n = 7	49 ± 3.71	48 ± 3.19**
	(C) n = 4	45 ± 4.02	46 ± 3.88**

All values are mean ± S.E.

*$P<0.05$

** not significant

Figure 17

Hypothalamic sites of stimulation. All sites inhibited noradrenaline induced reflex vagal bradycardia. For convenience sites stimulated during medullary stimulation are shown on the left and sites that elicited medullary field potentials are shown on the right. ○, sites that inhibited ipsilateral medullary bradycardia; ●, sites that inhibited contralateral medullary bradycardia; ■, sites eliciting field potentials in ipsilateral medulla; X, sites eliciting field potentials in contralateral medulla.

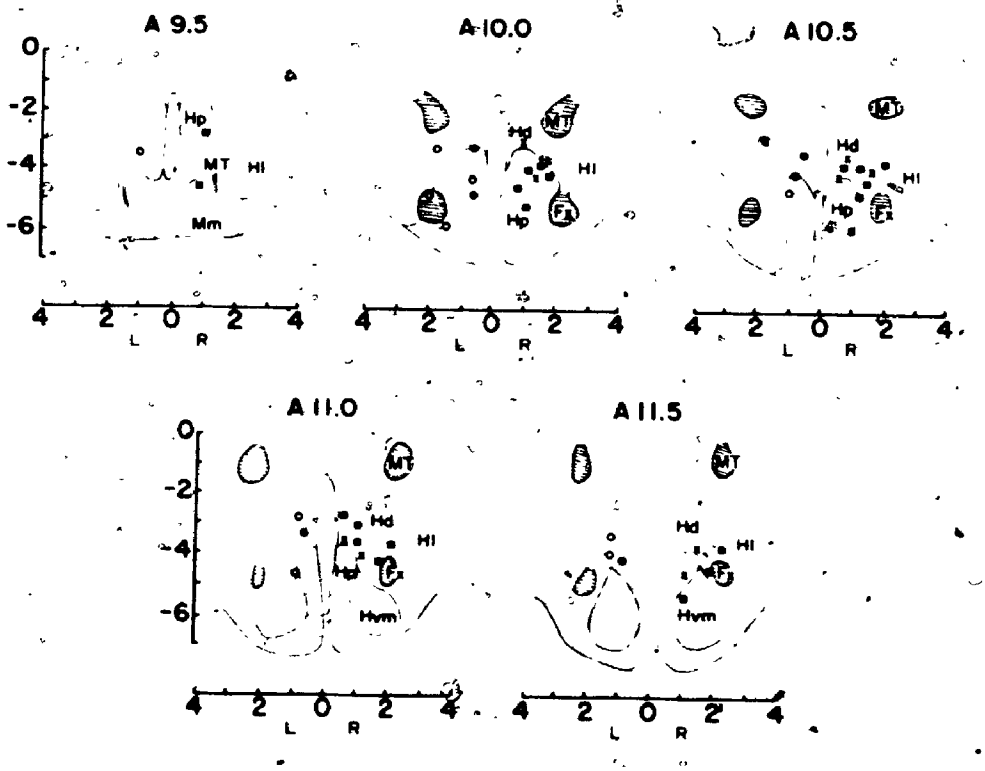

A 9.5
A 10.0
A 10.5
A 11.0
A 11.5
0
-2
-4
-6
Hp
MT
HI
Mm
Hd
Fx
Hvm
4 2 0 2 4
L
R

Figure 18

Medullary sites of recording and stimulation. For convenience, all stimulation sites are shown on the left and recording sites are shown on the right. ■, location of field potentials elicited by stimulation of ipsilateral posteromedial hypothalamus (PMH); ✖, location of field potentials elicited by stimulation of contralateral PMH; ▼, location of stimulating electrode at which vagal bradycardia was inhibited by simultaneous stimulation of the ipsilateral or contralateral PMH; ●, location of medullary vagal bradycardia that were not inhibited by ipsilateral or contralateral PMH stimulation. The transverse sections of the medulla are modified from the stereotaxic atlas of Berman (1968). For identification of structures see Figure 21. The number in each transverse section represents distance (mm) rostral (+) or caudal (-) to the obex.

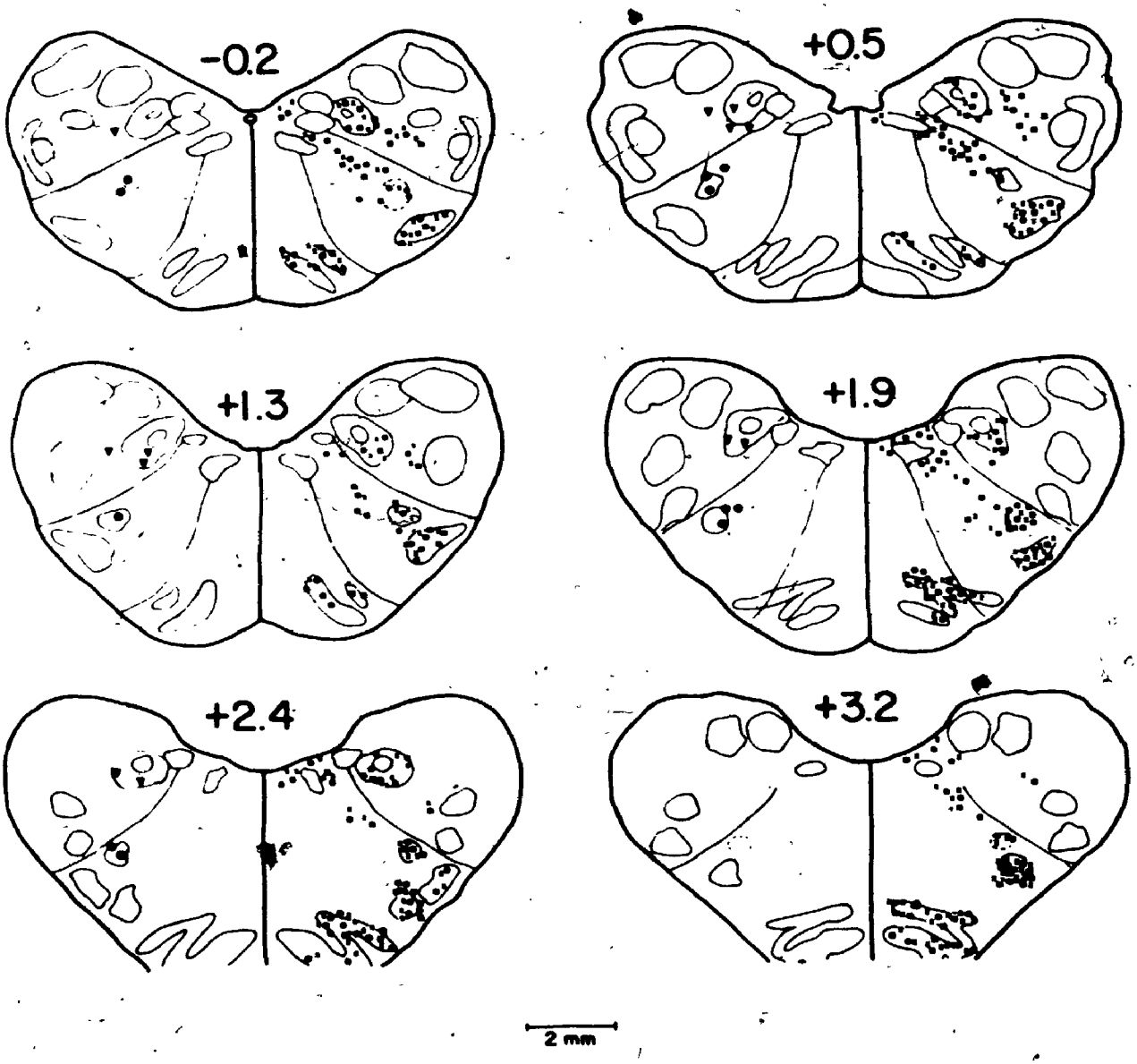
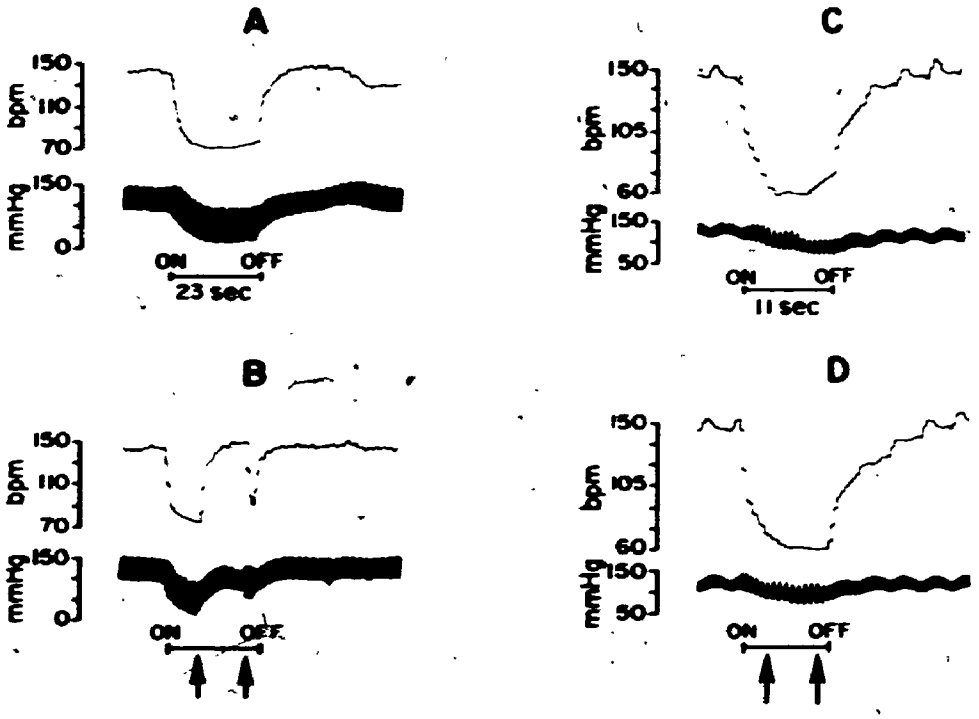

-0.2
+0.5
+1.3
+1.9
+2.4
+3.2
2 mm

Figure 19

Effect of stimulation of the posteromedial hypothalamus (PMH) on medullary vagal bradycardia elicited by stimulation of the n. of tractus solitarius (NTS) and n. ambiguus (NA). A, stimulation of NTS. B, simultaneous stimulation of NTS and PMH. C, stimulation of NA. D, simultaneous stimulation of NA and PMH. In each record, medullary stimulation represented by solid bar and PMH stimulation occurred between arrows. Note inhibition of NTS but not NA bradycardia.


A
150
110
70
bpm
150
0
mmHg
ON
OFF
23 sec
C
150
105
60
bpm
150
50
mmHg
ON
OFF
11 sec
B
150
110
70
bpm
150
0
mmHg
ON
OFF
D
150
105
60
bpm
150
50
mmHg
ON
OFF

stimulation in the PMH that inhibited the reflex vagal bradycardia elicited by intravenous administration of noradrenaline were used. In addition, the same parameters of stimulation (voltage and pulse duration) that inhibited reflex bradycardia were used to elicit field potentials and the sites of penetration in the medulla were restricted to a region which mediates vagal bradycardia (Fig. 14).

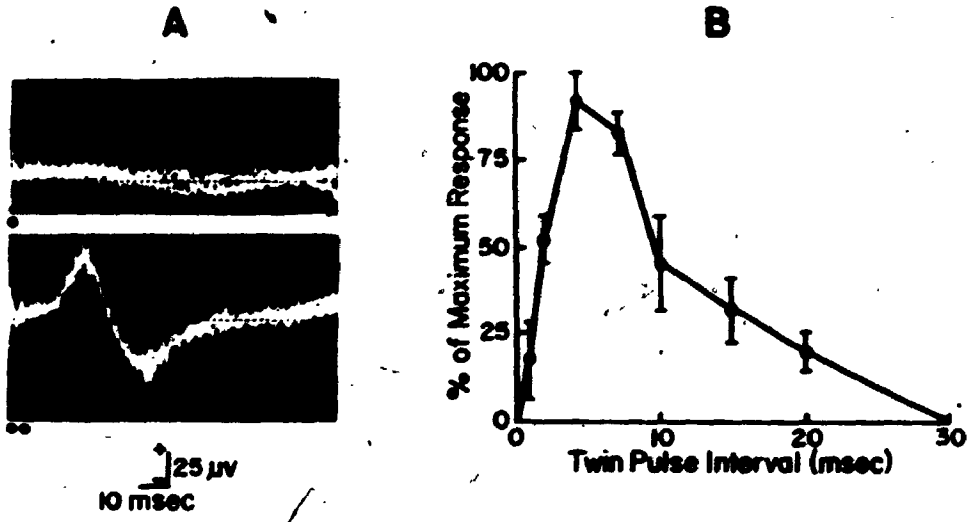
(a) Effect of single and twin pulse stimulation of the postero-medial hypothalamus on medullary field potentials

In the initial experiments the PMH was stimulated with single pulses and it was found that in four animals only nine medullary field potentials were recorded during 39 electrode penetrations. As previous investigations (Brookhart, 1952; Laursen and Wiesendanger, 1966) have shown that twin pulse stimulation can elicit responses that are absent during single pulse stimulation the possibility that the paucity of field potentials in the initial experiments was due to inadequate stimulation was investigated. It was found that the amplitude of field potentials was markedly increased during twin pulse stimulation of the PMH compared to the response elicited by single pulse stimulation; this is shown in Figure 20A. To determine the twin pulse interval that could elicit maximum responses at a constant voltage and pulse duration the effect of varying the pulse interval on the magnitude of field potentials was investigated systematically in six experiments and the results obtained are presented in Figure 20B. It can be seen that an increase in magnitude of the potentials was observed when the interval was between 1 and 30 msec, with the greatest increase occurring between

Figure 20

A. Effect of stimulation of the posteromedial hypothalamus (PMH) with twin pulses on a medullary field potential. Top, single pulse stimulation. Bottom, twin pulse stimulation, pulse interval 5 msec. Stimulus at dots. Note marked increase in amplitude during twin pulse stimulation.

B. Amplitude of evoked potentials versus twin pulse intervals. Points represent means, bars represent S.E., n = 6 potentials. Note marked increase in amplitude with twin pulse intervals between 2 and 10 msec.


A
B
25 µv
10 msec
100
75
50
25
0
% of Maximum Response
0
10
20
30
Twin Pulse Interval (msec)

2 and 10 msec. On the basis of these results in all successive experiments (17 animals) the PMH was stimulated with twin pulses with an interval of 5 msec.

(b) Location of medullary and hypothalamic sites of stimulation and of recording

Thirty-one histologically confirmed sites in the PMH at which stimulation inhibited reflex vagal bradycardia and elicited field potentials in the medulla were located in a discrete region of the hypothalamus medial to the fornix and extending from the rostral portion of the mammillary bodies to the caudal region of the ventromedial hypothalamic nucleus (Fig. 17).

The location of 405 histologically confirmed sites in the medulla at which field potentials were recorded is shown in Figure 18. It can be seen that almost all the field potentials were recorded from or near nuclear regions; the majority (382/405) were located in seven medullary nuclei: inferior olivary nucleus, 110; lateral reticular nucleus, 91; nucleus of tractus solitarius, 45; ventral nculeus medullae oblongatae centralis, 43; nucleus ambiguus, 38; parahypoglossal area, 29; and dorsal nucleus medullae oblongatae centralis, 26. The remaining 23 field potentials were recorded in a fibre tract, the dorsal longitudinal fasciculus, and in Figure 18 it can be seen that the majority of these sites were located in the rostral area of the medullary region under investigation. The distribution of sites of recording of field potentials is shown in Table 12. That the field potentials could be recorded more readily in

TABLE 12. Distribution of field potentials in the medulla elicited by stimulation of the posteromedial hypothalamus (PMH) in 21 cats.

	Stimulation of Ipsilateral PMH		Stimulation of Contralateral PMH		TOTAL	
	n	%	n	%	n	%
RIGHT MEDULLA						
ION	33	24.0	18	27.8	51	25.3
LRN	23	16.8	14	21.5	37	18.3
NTS	20	14.6	9	13.8	29	14.4
v-MOC	20	14.6	6	9.2	26	12.9
PHA	13	9.5	5	7.7	18	8.9
NA	13	9.5	3	4.6	16	7.9
d-MOC	9	6.6	5	7.7	14	6.9
DLF	6	4.4	5	7.7	11	5.4
TOTAL	137	100	65	100	202	100
LEFT MEDULLA						
ION	30	28.7	29	29.3	59	29.1
LRN	28	26.8	26	26.3	54	26.6
NA	11	10.5	11	11.0	22	10.8
v-MOC	9	8.6	8	8.1	17	8.4
NTS	8	7.6	8	8.1	16	7.9
d-MOC	6	6.4	6	6.1	12	5.9
DLF	6	5.7	6	6.1	12	5.9
PHA	6	5.7	5	5.0	11	5.4
TOTAL	104	100	99	100	203	100

DLF, dorsal longitudinal fasciculus; ION, inferior olivary n.; LRN, lateral reticular n.; NA, n. ambiguus; d and v-[redacted], dorsal and ventral n. medullae oblongatae centralis; NTS, n. of tractus solitarius; PHA, parahypoglossal area.

or near medullary nuclei is demonstrated in Figure 21 which shows a characteristic response recorded in the medulla as a function of depth of electrode penetration; it can be observed that the maximum amplitude of the potential was obtained as the electrode passed through a nuclear region.

(c) Characteristics of medullary field potentials elicited by stimulation of the posteromedial hypothalamus

The field potentials recorded in the medulla could be divided into two groups on the basis of their waveform: those in the first group (9% of the potentials) were monophasic (positive or negative) and those in the second group (91%) were biphasic (positive-negative, 185; negative-positive, 193). The mean latency to the peak of the first component of the field potential was 35 ± 3.1 msec (range 19-53 msec); the distribution of the latencies is summarized in Figure 22. In this study, medullary field potentials were recorded in order to investigate hypothalamo-medullary connections and no attempt was made to characterize medullary structures on the basis of the waveform of these potentials.

The possibility that the medullary potentials might have been due to movement artifacts induced by hypothalamic stimulation was investigated by administration of the muscle relaxant gallamine triethiodide (Flaxedil, Poulenc Laboratories, Montreal; 5 mg/kg, i.v.)

Figure 21

Amplitude of field potentials elicited in the medulla by stimulation of the posteromedial hypothalamus as a function of depth of penetration. Note maximum amplitude when electrode tip is in nuclear region. DLF, dorsal longitudinal fasciculus; XII, hypoglossal n.; ION, inferior olivary n.; LRN, lateral reticular n,; NA, n. ambiguus; NC, n. cuneatus; NG, n. gracilis; d- and v-MOC, dorsal and ventral n. medullae oblongatae centralis; NTS, n. of tractus solitarius; DX, dorsal n. of vagus; PHA, parahypoglossal area; SV, spinal n. of trigeminal nerve.

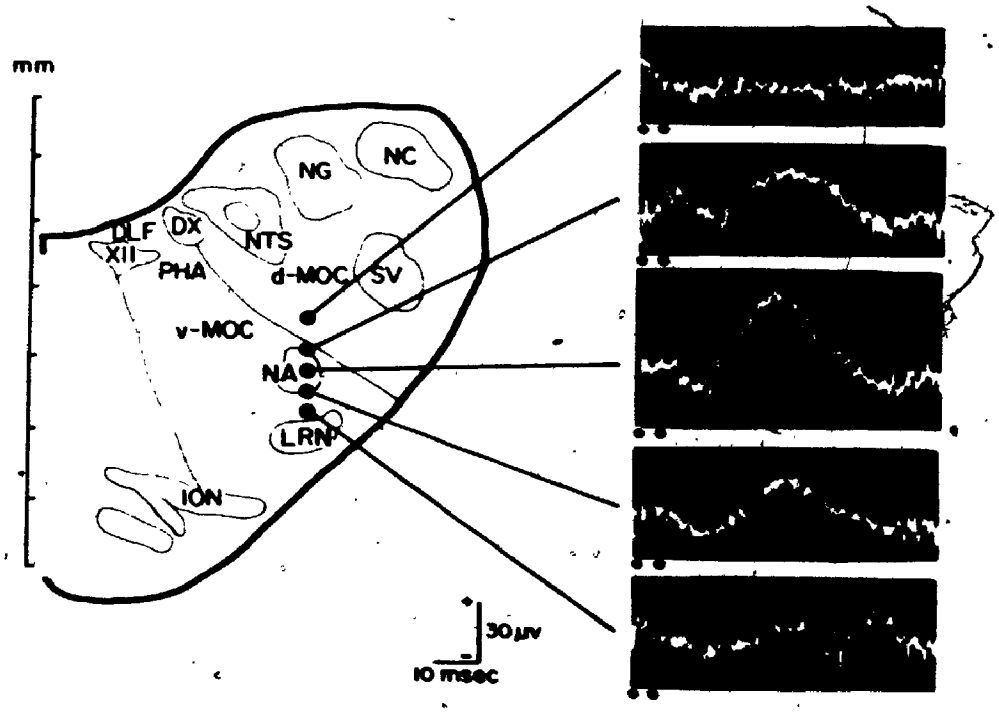
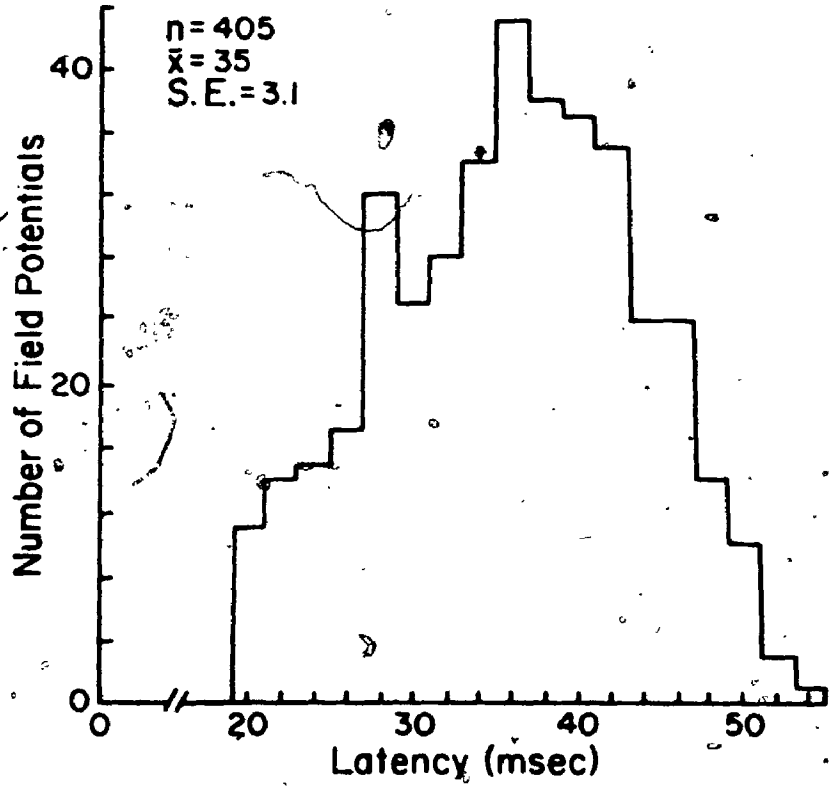

mm
NG
NC
DLF
DX
NTS
XII
PHA
d-MOC
SV
v-MOC
NA
LRN
ION
30 μv
10 msec

Figure 22

Histogram of latencies to peak response of medullary field potentials elicited during stimulation of the posteromedial hypothalamus.


n=405
x̄=35
S.E.=3.1
Number of Field Potentials
0
20
40
0
20
30
40
50
Latency (msec)

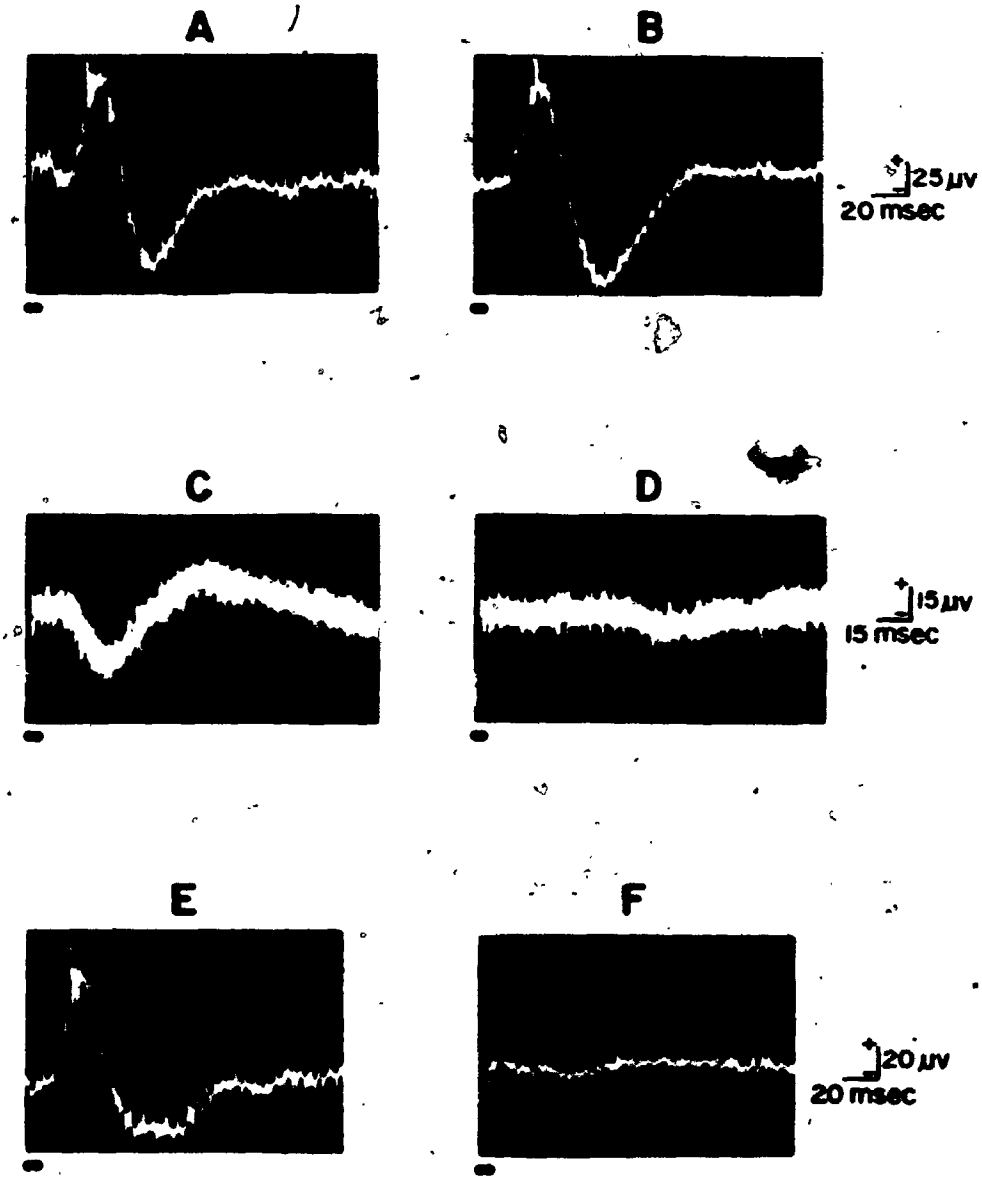
and it was found that the amplitude, latency and duration of 29 ipsilateral and 16 contralateral field potentials were not altered by the drug. Typical responses before and after gallamine administration are shown in Figure 23 A and B. In addition, the possibility that the medullary field potentials were elicited by antidromic stimulation of ascending fibres was investigated in the following experiments. First, at medullary sites from which field potentials could be recorded the effect on the response of different frequencies of stimulation was investigated and it was found that none of the potentials followed frequencies of stimulation higher than 26 Hz. Second, it was shown that in 15 animals 150 sec of asphyxia induced by cessation of artificial ventilation in paralyzed animals abolished 8 ipsilateral and 7 contralateral field potentials. Typical responses before and after asphyxia are shown in Figure 23, C and D. Third, in 6 animals, intravenous administration of sodium pentobarbital (20 mg/kg, Nembutal, Abbott Laboratories, Montreal) abolished 3 ipsilateral and 3 contralateral field potentials; typical responses before and after sodium pentobarbital administration are shown in Figure 23, E and F.

(d) Effect of pontomedullary transection on medullary field potentials elicited by stimulation of the posteromedial hypothalamus

The location of the descending pathways mediating the medullary field potentials induced by stimulation of the PMH was investigated by comparing the effect of hemitransection and bilateral transection of the brain stem at the pontomedullary level on the amplitude of the

Figure 23

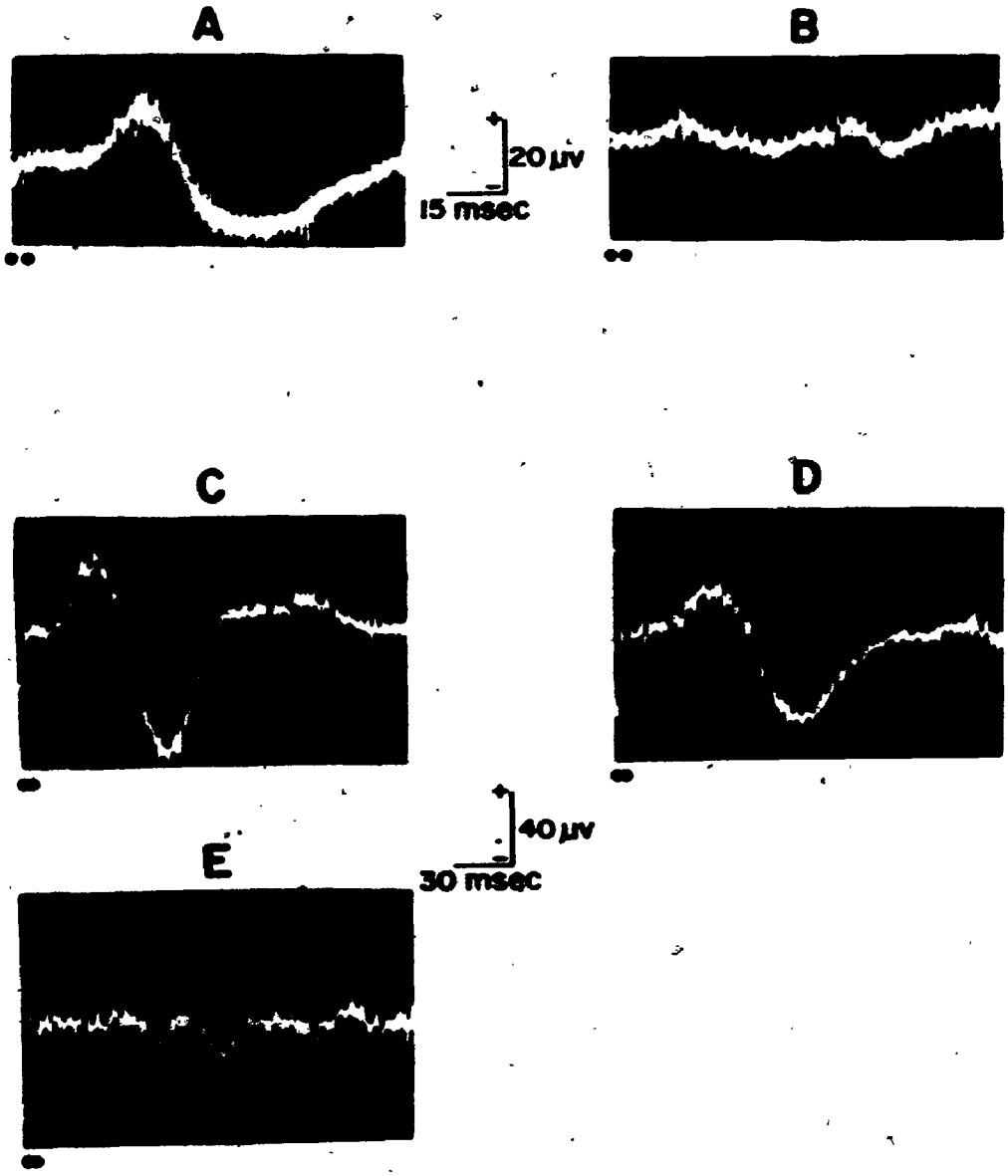
Effect of various experimental procedures on medullary field potentials. A, B: before and after gallamine triethiodide (5 mg/kg i.v.); C, D: before and after asphyxia (150 sec); E, F: before and after sodium pentobarbital (20 mg/kg i.v.).


A
B
25 µv
20 msec
C
D
15 µv
15 msec
E
F
20 µv
20 msec

field potential in six cats. Six ipsilateral field potentials (3 in the right and 3 in the left medulla) were consistently abolished by ispilateral hemitransection at the pontomedullary junction. However, ipsilateral transection only reduced the amplitude of one contralateral field potential in the right medulla and 3 contralateral field potentials in the left medulla and bilateral transection was required to abolish these contralateral responses. The characteristic response to transection is shown in Figure 24 in which it can be seen that ipsilateral field potentials were completely abolished by ipsilateral hemitransection (Figure 24 A and B) and that contralateral field potentials were only reduced by ipsilateral hemitransection (Figure 24, C and D) and abolished by bilateral transection (Figure 24, E).

Figure 24

Effect of ipsilateral transection of the brain stem at the pontomedullary level on the amplitude of medullary field potentials elicited by stimulation of the ipsilateral (A, B) and the contralateral (C, D, E) posteromedial hypothalamus. Note that ipsilateral transection abolished the ipsilateral field potential (B) but only reduced the amplitude of the contralateral field potential (D), which was abolished by bilateral transection (E).


A
B
20 µv
15 msec
C
D
40 µv
30 msec
E

DISCUSSION

The aim of this project was to study the role of a well defined hypothalamic region in the control of cardiovascular reflexes and to investigate the mechanism(s) involved in this control. A discrete vasomotor region was identified in the posteromedial hypothalamus (PMH) and baroreceptor and chemoreceptor inputs were shown to project to it. In addition, electrical stimulation of the PMH was found to inhibit the vagal component of the baroreceptor and chemoreceptor reflexes. Furthermore, medullary structures mediating reflex vagal bradycardia were identified and stimulation of the PMH was shown to inhibit the vagal bradycardia elicited by medullary stimulation. Finally a projection from the PMH to these medullary structures was demonstrated electrophysiologically.

The discussion will consist of an analysis of the detailed experimental findings and the conclusions that may be drawn from these observations with regard to neural control of the cardiovascular system.

A. Discussion of experimental findings

(a) Cardiovascular response to stimulation of the posteromedial hypothalamus

Many of the previous investigations of the cardiovascular responses elicited by electrical stimulation of the hypothalamus based

on the use of stereotaxic coordinates and the histological appearance of electrode tracts to localize the sites of stimulation (Folkow & Rubinstein, 1966; Gebber & Snyder, 1970; Hilton & Spyer, 1968; Ninomiya, Judy & Wilson, 1970) have failed to provide precise localization of cardiovascular function in discrete regions in the hypothalamus. In the present investigation, the histological localization of the electrode tip has permitted the accurate determination of the sites of stimulation and the investigation of a discrete hypothalamic region with vasomotor function.

Electrical stimulation of this discrete vasomotor area located in the posteromedial hypothalamus has been found to elicit marked increases in heart rate and arterial pressure. This cardiovascular response is due to sympathetic excitation since the cardioacceleration is not affected by vagotomy but is abolished by administration of propranolol. However, as the animals in these experiments were under alpha-chloralose anaesthesia the possibility that electrical stimulation of this area may also inhibit the vagal input to the heart could not be categorically excluded in view of the recent demonstration that central vagal activity is depressed by alpha-chloralose (Calaresu & Thomas, 1971).

(b) <u>Baroreceptor and chemoreceptor inputs to the posteromedial hypothalamus</u>

The results of previous studies showing that hypothalamic neural activity was influenced by baroreceptor and chemoreceptor activation are difficult to interpret because the parameters of the

stimuli employed to excite baroreceptors and chemoreceptors (Baust & Katz, 1961; Baust, Niemeyzk, Schaeffer & Vieth, 1962; Cross, 1964; Cross & Silver, 1963; Frazier, Taquini, Boyarski & Wilson, 1965; Hilton & Spyer, 1968) were such that changes in blood flow to the hypothalamus and alterations in the blood gases perfusing the brain would be expected. In the present investigation the problem of eliciting systemic cardiovascular changes by electrical stimulation of the carotid sinus nerve (CSN) was circumvented by employing stimulation of the CSN at such a frequency that no changes in systemic cardiovascular parameters were observed. This technique, combined with the use of the post-stimulus time (PST) histogram which summates the discharges of single neurones after a stimulus, has allowed the demonstration of excitatory and inhibitory responses to CSN stimulation without altering heart rate or arterial pressure. In addition, with these techniques it was possible to determine latency and duration of the responses.

The activity of 51 single units located in the posteromedial hypothalamus was recorded during electrical stimulation of the CSN. The paucity of active and responsive units obtained in this investigation may have been due to the use of alpha-chloralose as it has been shown that this drug greatly decreases the number of spontaneously active hypothalamic units and their responsiveness to afferent stimulation (Stuart, Porter, Adey & Kamikawa, 1964). The 23 units that were excited or inhibited by CSN stimulation were found to be located in the same posteromedial region of the hypothalamus from which electrical stimulation produced cardioacceleration and arterial hyper-

tension. In contrast, the majority of the 28 units that did not alter their firing frequency were found in areas that were either ventral or lateral to the location of the responsive units.

With regard to the specificity of the responses of these units to CSN stimulation, although evoked potentials and changes in the discharge frequency of single units have been elicited in the hypothalamus of cats and rats by somatosensory, light and acoustic stimulation (Dafny, Bental & Feldman, 1965; Dafny, Peritz, Fischler & Feldman, 1970; Rudomin, Malliani & Zanchetti, 1965; Sarne & Feldman, 1971; Stuart et al, 1964), the demonstration in the present investigation that selective stimulation of baroreceptors and chemoreceptors altered the activity of these units strongly suggests that the excitatory and inhibitory responses elicited in the posteromedial hypothalamus by stimulation of the CSN were due to activation of baroreceptor and chemoreceptor fibres. Furthermore, it may be concluded on the basis of the broad ranges of latency and duration of the responses elicited by CSN stimulation that the baroreceptor and chemoreceptor inputs are mediated by polysynaptic pathways (Miura & Reis, 1969). Finally, as only ipsilateral CSN stimulation was attempted in this study, the possibility that the afferent pathways may also ascend to the contralateral side cannot be excluded, particularly in view of the demonstration of contralateral representation of baroreceptor input at the medullary level (Calaresu & Pearce, 1965b; Crill & Reis, 1968).

As it has been shown that the CSN contains baroreceptor and chemoreceptor A-fibres (Fidone & Sato, 1969) the demonstration of inhibition of some hypothalamic units and of excitation of others by

stimulation of the CSN prompts the suggestion that the two responses were due to activation of baroreceptor and chemoreceptor fibres, respectively. The observation that the activity of some units was inhibited by stimulation of the CSN and by noradrenaline (NA) injection, but not by injection of sodium cyanide (NaCN), adds evidence to the suggestion that the inhibitory response was due to excitation of baroreceptor afferent input. In addition, the failure to observe any response to NA or NaCN injection in those units that were not affected by CSN stimulation (Table 3) provides additional evidence that the response to NA injection was mediated by excitation of peripheral receptors and was not due to a direct effect of the drug on the cell or to the changes in blood flow in the hypothalamus that occurred during the NA induced hypertension.

With regard to the seven units that were excited by CSN stimulation it may be suggested that these units under physiological conditions are excited by activation of chemoreceptors. If this suggestion is correct, these units should be excited by selective stimulation of carotid body chemoreceptors by NaCN injection. This proved extremely difficult to demonstrate as it required finding a hypothalamic unit that was excited by CSN stimulation in an animal in which the carotid body chemoreceptors were responsive to NaCN injection. However, in one animal in which carotid chemoreceptors were responsive to NaCN injection one unit was found which was excited by CSN stimulation and whose discharge frequency was increased markedly during NaCN induced bradycardia (Table 3 and Figure 6). This observation supports the hypothesis that the excitatory response elicited in the posteromedial hypothalamus by stimulation of the CSN, was due to excitation of

chemoreceptor fibres.

If it is accepted that the inhibitory response was due to excitation of baroreceptor fibres and that the excitatory response was due to stimulation of chemoreceptor fibres, an attempt can be made to assign a physiological role to these hypothalamic units. It is well documented that baroreceptor activation elicits arterial hypotension by sympathetic inhibition (Hainsworth, Ledsome & Carswell, 1970; Heymans & Neil, 1958; Kezdi & Geller, 1968) and that stimulation of chemoreceptors elicits arterial hypertension by sympathetic excitation (Biscoe, 1971; Comroe & Mortimer, 1964; Heymans & Neil, 1958; Neil, 1956). The baroreceptor and chemoreceptor induced changes in sympathetic activity are generally considered to be mediated by changes in the activity of tonic sympathetic cells located in the medulla and pons (Uvnäs, 1960). However, it has recently been suggested that cardiovascular reflexes initiated by baroreceptor activation may also be mediated by supramedullary structures (Peiss, 1965). This hypothesis is supported by the demonstration that the amplitude of the pressor response elicited in cats by carotid occlusion is significantly reduced after midcollicular decerebration (Reis & Cuénod, 1965). In addition, the observation that the pressor response to carotid occlusion elicited in cats with extensive medullary lesions is abolished by decerebration (Manning, 1965) clearly demonstrates that neural mechanisms concerned with cardiovascular reflexes are not confined to medullary centres. In the present investigation, the observation that electrical stimulation of the CSN and selective excitation of baroreceptors inhibits unit activity in a region of the hypothalamus electrical

stimulation of which elicits hypertension and cardioacceleration provides direct evidence for the involvement of supramedullary regions in cardiovascular reflexes. In addition, these results prompt the suggestion that this hypothalamic area mediates the supramedullary component of the pressor response to carotid occlusion (Manning, 1965) as removal of the inhibitory baroreceptor input to this region during carotid occlusion would result in an increase in the activity of a region which has been shown by electrical stimulation to produce arterial hypertension and cardioacceleration.

(c) Cardiovascular response to chemoreceptor stimulation

This study demonstrates that the bradycardia elicited by intracarotid injection of sodium cyanide (NaCN) is due solely to excitation of the vagal input to the heart as the cardiac slowing was not significantly altered either by intravenous administration of propranolol or by transection of the spinal cord at C_7 but was abolished by bilateral cervical vagotomy. In addition, the persistence of a reduced bradycardia after right cervical vagotomy demonstrates that the reflex cardiac slowing elicited by excitation of right carotid body chemoreceptors is mediated by both vagi.

An interesting finding was the failure to observe a cardioacceleration secondary to the chemoreceptor induced hyperventilation. These results are at variance with previous studies in which the primary reflex bradycardia was obscured by the cardioacceleration elicited during hyperventilation (Daly & Scott, 1958; Scott, 1966). This cardioacceleration may have been due to a decrease in the

spontaneous vagal discharge as inspiration has been shown to inhibit spontaneous vagal activity in the dog (Katona, Poitras, Octo Barnett & Terry, 1970). In the present investigation, however, a decrease in vagal activity during chemoreceptor induced hyperventilation is unlikely to have occurred as it has been shown that spontaneous vagal discharge to the heart is probably absent in chloralosed cats (Calaresu & Thomas, 1971).

Although it may be argued that perfusion of the carotid body with hypoxic blood is a more physiological stimulus to chemoreceptors than intracarotid injection of NaCN, this drug was used in this study as it is well documented that carotid body chemoreceptors are selectively excited by NaCN. This has been demonstrated by the finding that the discharge frequency of carotid sinus baroreceptor fibres is not affected by intracarotid injection of NaCN when given in doses sufficient to increase the frequency of discharge of chemoreceptor fibres and to produce marked bradycardia (Jacobs, Sampson & Comroe, 1971). In addition, although intravenous administration of large doses of NaCN (0.3 mg/kg) in sino-aortic denervated dogs has been shown to elicit cardioacceleration and arterial hypertension (Krasney, 1970), the possibility that the cardiovascular changes observed in the present investigation were due to a direct stimulation of structures in the central nervous system is unlikely as it has been demonstrated that the bradycardia and the arterial hypertension elicited by intracarotid injection of 50 µg of NaCN in the dog is abolished by section of the carotid sinus nerve (CSN) (Jacobs et al, 1971). Similarly, in two of the experiments reported here it was shown that section of the CSN

abolished the cardiac slowing and the arterial hypertension elicited by intracarotid administration of NaCN.

The demonstration that the chemoreceptor induced bradycardia was due solely to excitation of the vagal input to the heart is at variance with previous observations in bilaterally vagotomized dogs (Daly & Scott, 1958) and cats (MacLeod & Scott, 1964), in which cardiac slowing due to sympathetic inhibition occurred during perfusion of the isolated carotid sinus with hypoxic blood. A possible explanation for these conflicting results may be found in the method used in these investigations to excite chemoreceptors. Prolonged perfusion of the carotid sinus with hypoxic blood may have excited baroreceptors; this is a likely possibility as baroreceptors can be activated after a delay of 10 sec by doses of NaCN larger than those required to excite chemoreceptors (Jacobs et al, 1971); this delay has been attributed to the relative insensitivity of baroreceptors to chemical agents (Jacobs et al, 1971) based on the anatomical finding that baroreceptors are located in the adventitia of the carotid artery and receive a relatively sparse blood supply (Heymans & Neil, 1958). If it is accepted that NaCN mimics the effects of hypoxia, the possibility exists that the cardiac slowing previously observed in vagotomized animals during prolonged perfusion of the isolated carotid sinus may have been due to excitation of baroreceptors which is known to inhibit the sympathetic input to the heart (Green & Heffron, 1968; Scher & Young, 1970; Thames & Kontos, 1970).

This study has demonstrated that the reflex bradycardia obtained by stimulation of the right carotid body is not mediated predominantly by the ipsilateral vagus. These results are different

from the results of previous studies in the dog (McQueen & Ungar, 1971) and may be due to a species difference.

The failure to observe a significant change in the amplitude of the chemoreceptor induced bradycardia after spinal transection, which abolishes the pressor response to chemoreceptor stimulation, clearly demonstrates that the reflex vagal bradycardia is due solely to excitation of carotid body chemoreceptors and not to secondary activation of baroreceptors by the chemoreceptor induced arterial hypertension. In addition, the absence of bradycardia in the presence of a marked pressor response in vagotomized animals (Table 4, 6; Figure 7, 9) is an interesting finding and suggests the possibility that chemoreceptor excitation may decrease the sensitivity of the baroreceptor reflex to changes in arterial pressure. If this suggestion is accepted, the inhibition of the baroreceptor reflex observed during muscular activity (Bristow, Brown, Cunningham, Howson, Strange, Peterson, Pickering & Sleight, 1971; Coote, Nilton & Perez-Gonzalez, 1971) may in part be due to the increase in carotid body chemoreceptor discharge which has been shown to occur during movement of the hindlimbs (Biscoe & Purves, 1967b).

The observation that carotid body chemoreceptor activation elicits an increase in arterial pressure without increasing the sympathetic input to the heart demonstrates that different functional components of the sympathetic nervous system may be activated selectively and supports previous suggestions of a functional separation of areas in the central nervous system influencing the sympathetic discharge to the heart and blood vessels (Downing, Remensnyder & Mitchell, 1962;

Downing & Siegel, 1963).

(d) Hypothalamic inhibition of reflex vagal bradycardia

The finding that the magnitude of the chemoreceptor induced bradycardia was significantly increased after midcollicular decerebration strongly suggested that structures rostral to this level of transection inhibit the vagal component of the chemoreceptor reflex. The experiments reported here demonstrate that one of the sites of origin of this inhibitory influence is a discrete region of the posteromedial hypothalamus, electrical stimulation of which has been shown to inhibit the vagal bradycardia elicited by selective excitation of right carotid body chemoreceptors. In addition, the observation that the bradycardia elicited by stimulation of the right carotid sinus nerve (CSN) or by activation of chemoreceptors in the right carotid body was inhibited by electrical stimulation of the ipsilateral and contralateral posteromedial hypothalamus (PMH) demonstrates that this inhibitory influence is mediated by crossed as well as uncrossed pathways.

Although it is well documented that electrical stimulation of the hypothalamus inhibits the vagal component of the baroreceptor reflex (Djojosugito, Folkow, Kylstra, Lisander & Tuttle, 1970; Gebber & Snyder, 1970; Hilton, 1963; Humphreys & Joels & McAllan, 1971) the absence in these previous studies of precise localization of the sites of stimulation has failed to provide information regarding discrete inhibitory regions in the hypothalamus. In the present investigation, however, the histological localization of the electrode tip

has permitted the accurate determination of the sites of stimulation and has demonstrated for the first time the existence of a discrete region in the PMH electrical stimulation of which inhibits vagal bradycardia induced by excitation of both baroreceptors and chemoreceptors. It is recognized that other hypothalamic regions may have an influence on cardiovascular reflexes mediated by medullary mechanisms. However, the findings reported here combined with the demonstration that baroreceptor and chemoreceptor inputs project to this hypothalamic region strongly suggest that the PMH has an important role in the control of reflex vagal bradycardia.

(e) Medullary structures mediating vagal bradycardia

In this study vagal cardioinhibitory sites have been accurately localized to discrete regions of the medulla of the cat. The results observed were due solely to excitation of the vagus as the changes in heart rate were obtained in animals transected at C_7 which interrupts descending sympathetic pathways to the heart. The possibility that the cardiovascular responses were secondary to respiratory changes observed during medullary stimulation may be excluded as a consistent cardiac response was elicited in the presence of inconsistent respiratory responses and cardiac slowing could be elicited in artificially ventilated animals by electrical stimulation of comparable locations.

The accurate histological identification of the sites of stimulation has demonstrated the existence of several medullary structures possessing a cardioinhibitory function. In agreement with previous observations electrical stimulation of the nucleus of tractus solitarius

(NTS) (Achari, Downman & Weber, 1968; Calaresu & Pearce, 1965b; Gunn, Sevelius, Puiggari & Myers, 1968; Quest & Gebber, 1972; Seller & Illert, 1969) and the nucleus ambiguus (NA) (Gunn et al, 1968; Quest & Gebber, 1972) has been found to produce a vagal bradycardia. In addition to these previously identified cardioinhibitory areas, in the present investigation the dorsal and ventral n. medullae oblongatae centralis (MOC), the n. tractus spinalis trigemini (5SP), the intramedullary rootlets of the vagus nerve and the lateral reticular nucleus (LRN) have been shown to possess a cardioinhibitory function. All these regions are located in the lateral reticular formation, which is not surprising as it has been shown that the vagal response to baroreceptor excitation is not abolished by destruction of the medial reticular formation (Smith, Humphrey & Nathan, 1965). In addition, 12 sites were located in a dorsomedial region between the hypoglossal and ventral MOC nuclei. This region does not correspond to a well defined anatomical structure and could be considered a rostral extension of an area previously implicated in the regulation of heart rate (Calaresu & Henry, 1970). With regard to the possible mechanism of the bradycardia elicited by stimulation of the ventral MOC, the 5SP and the LRN it can only be suggested that these nuclei may provide an excitatory input to cardioinhibitory neurones. This suggestion is supported by the demonstration that the LRN receives a cardiovascular input from the hypothalamus (Smith, 1965) and from the aortic depressor nerve (Kumada & Nakajima, 1972); similarly it has been shown that the MOC receives inputs from the NTS (Morest, 1967) and from the carotid sinus nerve (Miura & Reis, 1969); finally, the 5SP receives

afferent fibres from the trigeminal nerve electrical stimulation of which has been shown to induce efferent activity in the vagus nerve and slowing of the heart (Green, DeGroot & Sutin, 1957).

The data obtained from this series of experiments regarding the effect of section of the vagus nerves on the medullary bradycardia differ in some respects from those of previous studies. The abolition of the bradycardia elicited by stimulation of the n. ambiguus (NA) by ipsilateral vagotomy is at variance with a previous report that ipsilateral vagotomy only reduced and that bilateral vagotomy was required to abolish the cardiac slowing elicited by stimulation of the NA (Gunn et al, 1968). This discrepancy may be due to the method and parameters used to stimulate the NA. In the present investigation small tip (5 - 10μ) unipolar electrodes elicited a bradycaria without accompanying motor activity whereas in the previous investigation bipolar electrodes (tip separation 0.5 mm) elicited a bradycardia that was accompanied by panting or coughing, and ipsilateral movements of the jaw and neck. A similiar discrepancy exists concerning the effect of ipsilateral vagotomy on NTS bradycardia. In this study ipsilateral vagotomy abolished NTS bradycardia while in a previous investigation it has been reported that the NTS bradycardia persisted after ipsilateral vagotomy (Calaresu & Pearce, 1965b). At present no explanation for this discrepancy can be given as the sites of stimulation in both experiments appears to be comparable. However, it is possible that the five sites of NTS stimulation reported in the previous paper (Calaresu & Pearce, 1965b) may have been located in the small medial extension of this nucleus merging with the contralateral NTS (commissure nucleus, Cottle, 1964;

Taber, 1961), whereas in the experiments reported here the NTS sites were all rostral to the commissure nucleus.

It has been reported that electrical stimulation of the paramedian reticular nucleus (Calaresu & Thomas, 1971) and of the nucleus intercalatus (Calaresu & Henry, 1970) elicits cardioacceleration due to inhibition of efferent vagal activity. It is therefore interesting that in the present investigation after ipsilateral vagotomy stimulation of the NTS, ventral MOC and NA also elicited cardioacceleration, which was due to an inhibition of the efferent vagal activity in the contralateral vagus as it was present in the spinal animal and was abolished by bilateral vagotomy. Although electrical stimulation of the NA in the sympathectomized atropinized dog has been reported to produce a cardioacceleration (Weiss & Priola, 1972) because this response was abolished by ipsilateral vagotomy it is very unlikely that the cardioacceleration was mediated by the same mechanism postulated for the cardioacceleration described in this study.

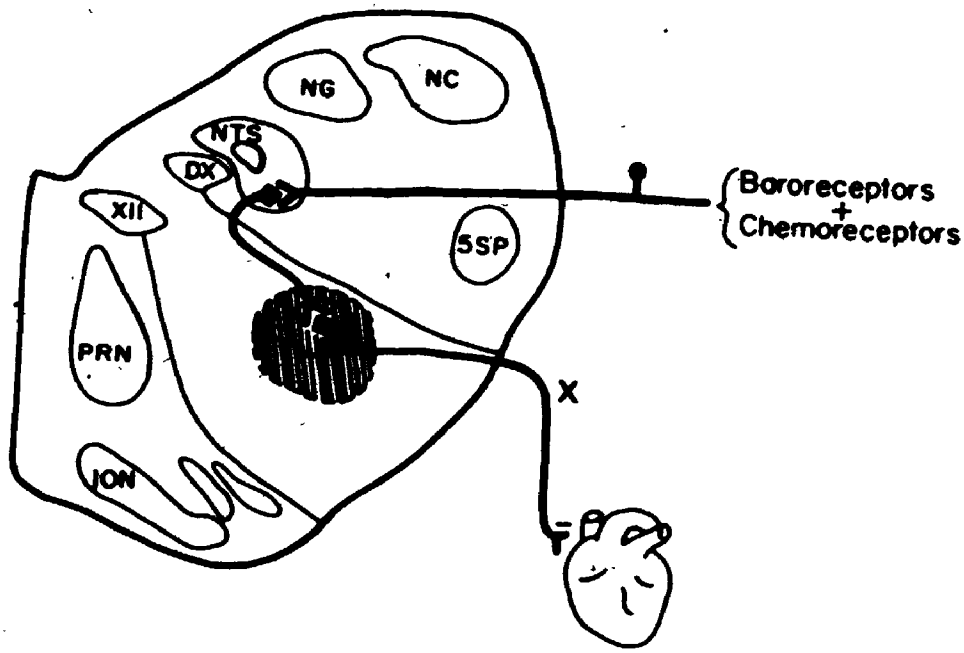
The finding that administration of sodium pentobarbital decreased the cardioinhibitory effect of peripheral vagal stimulation is in agreement with previous findings that this anaesthetic agent is vagolytic and exerts a depressant action on the SA node (Cox, 1972). Although the magnitude of the bradycardia elicited from different medullary structures was essentially the same and the effect of vagotomy on the medullary bradycardia was the same regardless of the site of stimulation the differential effects of sodium pentobarbital on the bradycardia elicited by stimulation of different medullary structures suggests a possible functional role for these various medullary areas. As it was shown that the bradycardia elicited from the NA was as resistant to the

depressant effects of sodium pentobarbital as the bradycardia elicited by stimulation of the peripheral vagus it is suggested that the NA bradycardia was probably due to excitation of efferent fibres. On the other hand, the pronounced inhibitory effect of sodium pentobarbital on NTS bradycardia suggests that this cardiac slowing was due to stimulation of afferent fibres or possibly interneurones. Support for this suggestion is the demonstration that sciatic nerve stimulation which inhibits reflex vagal bradycardia but not the cardiac slowing due to stimulation of the peripheral vagus, inhibits NTS but not NA vagal bradycardia (Quest & Gebber, 1972).

On the basis of evidence obtained in this study and results in the literature a scheme illustrating the possible central pathways mediating baroreceptor and chemoreceptor reflex vagal bradycardia is presented in Figure 25. In this scheme, baroreceptor and chemoreceptor afferent fibres pass through the region of the dorsal MOC to terminate in the NTS. Carotid sinus and aortic depressor nerve afferent fibres have in fact been localized in this region of the dorsal MOC (Crill & Reis, 1968) and the suggestion that these afferent fibres relay in the NTS is based on anatomical (Cottle, 1964; Ingram & Dawkins, 1945; Kerr, 1962) and electrophysiological studies (Biscoe & Sampson, 1970; Crill & Reis, 1968; Humphrey, 1967; Miura & Reis, 1969; 1972; Seller & Illert, 1969). The projection of second order neurones from the NTS to the NA is based on the anatomical findings of Morest (1964): after lesioning the NTS he observed degenerated axons projecting ventrolaterally and preterminal degeneration in the region of the NA. According to this scheme the cardiac slowing elicited by stimulation

Figure 25

Schematic illustration of the suggested central pathways mediating reflex vagal bradycardia (for details see text). For identification of structures see Figure 13.


NG
NC
NTS
DX
XII
SSP
PRN
X
ION
Baroreceptors
+
Chemoreceptors

of the dorsal MOC was due to excitation of afferent baroreceptor and chemoreceptor fibres, the NTS bradycardia was due to excitation of second order neurones, the ventral MOC bradycardia was due to excitation of axons from the NTS travelling in the ventral MOC, and the bradycardia elicited from the region of the NA was due to excitation of efferent vagal cardioinhibitory neurones.

(f) Hypothalamic inhibition of medullary bradycardia

The results of this study show for the first time that electrical stimulation of the posteromedial hypothalamus (PMH) can inhibit the vagal bradycardia elicited by direct stimulation of some medullary cardioinhibitory sites. The finding that PMH stimulation inhibited the bradycardia elicited by stimulation of the nucleus of tractus solitarius (NTS) and of the dorsal part of the nucleus medullae oblongatae centralis (D-MOC) but failed to alter the vagal bradycardia elicited from the nucleus ambiguus (NA) provides additional support for the suggestion that the NTS and the dorsal MOC serve as major relay sites in the mediation of reflex vagal bradycardia and that the NA is probably the site of origin of cardioinhibitory neurones. The finding that electrical stimulation of both sides of the PMH inhibited the bradycardia elicited by stimulation of the right medulla demonstrates that the inhibitory effects of PMH stimulation are mediated by crossed as well as uncrossed pathways. However, this study provides no information regarding the site of crossing of the inhibitory pathway.

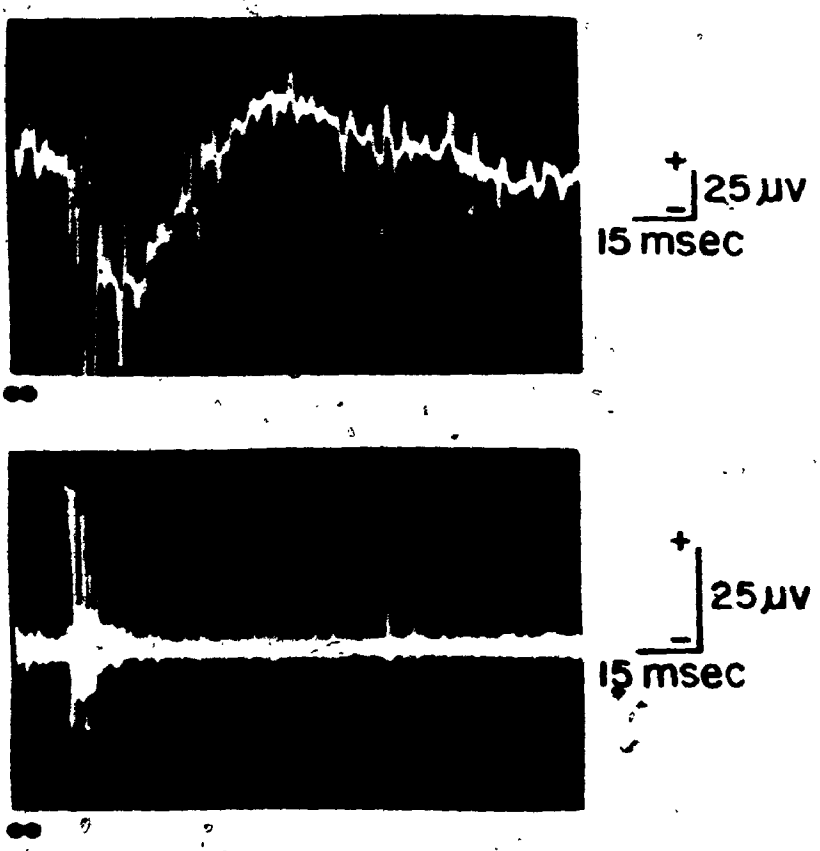
(g) Medullary field potentials elicited by stimulation of the posteromedial hypothalamus

The observation that the field potentials did not follow frequencies of stimulation greater than 26 Hz and that the responses could be abolished by asphyxia or intravenous administration of sodium pentobarbital but not by gallamine demonstrates that the potentials elicited during PMH stimulation were not due to movement artifacts but were due to excitation of a pathway synaptically connected with medullary structures. On the basis of latencies of 19 to 53 msec and of a conduction distance estimated at approximately 25 mm and if synaptic delays are considered negligible the conduction velocity of the fibres in this pathway may be estimated to be 0.5 to 1.3 meters/sec, indicating that this hypothalamo-medullary connection consists of small fibres. In addition, it is suggested that the field potentials were due to changes in the activity of discrete medullary nuclei because almost all potentials were recorded in medullary nuclear regions and on many occasions single unit activity was found to be in phase with a component of the field potential. An example of this relationship is presented in Figure 26 in which the unit activity can be seen to be temporally related to the negative component of the field potential.

The results of this study suggest that the hypothalamic inhibition of medullary vagal bradycardia is mediated by crossed and uncrossed descending pathways which project to and alter the activity in discrete medullary nuclei, as demonstrated by the observation that ipsilateral field potentials were completely abolished by ipsilateral

Figure 26

Field potential (top) and single unit activity (bottom) elicited at the same medullary site by stimulation of the posteromedial hypothalamus. Preamplifier bandpass 3-3000 Hz (top) and 300-3000 Hz (bottom).


+
25 µv
15 msec
+
25 µv
15 msec

hemitransection at the pontomedullary junction while contralateral field potentials were only reduced by ipsilateral hemitransection and abolished by bilateral transection. The suggestion that the field potentials were mediated by descending pathways is based on the observation that in 11 experiments reversing the electrodes (recording at the hypothalamic site of stimulation and stimulating at the medullary site of recording) failed to elicit any evoked activity.

On the basis of evidence in the literature and the results obtained in this study the functional role of several medullary structures in the mediation of hypothalamic induced changes in heart rate and arterial pressure may be suggested. With regard to changes in heart rate due to parasympathetic activation it is well documented that electrical stimulation of the hypothalamus inhibits reflex vagal bradycardia (Djojosugito, Folkow, Kylstra, Lisander & Tuttle, 1970; Gebber & Snyder, 1970; Hockman & Talesnik, 1971; Hilton, 1963; Humphreys, Joels & McAllen, 1971). In particular, the PMH has been shown in the present investigation to inhibit the vagal bradycardia elicited by selective excitation of baroreceptors and chemoreceptors. As the dorsal and ventral MOC, the NTS and the NA were shown to mediate vagal bradycardia and the PMH was found to project to these medullary regions the possibility exists that the hypothalamic induced inhibition of reflex vagal bradycardia may occur in these medullary nuclei. Although the synaptic events responsible for this inhibitory effect are unknown one possible mechanism may be presynaptic inhibition as it has been reported that hypothalamic stimulation produced primary afferent

depolarization in the carotid sinus nerve fibres terminating in the NTS (Weiss & Crill, 1969). The inferior olivary nucleus (ION) may also be involved in the mediation of hypothalamic inhibition of reflex vagal bradycardia as the PMH was shown to project to the ION and electrical stimulation of the ION has been reported to inhibit the carotid sinus reflex (Smith and Nathan, 1966).

With regard to changes in heart rate and arterial pressure due to sympathetic activation, it is well documented that hypothalamic stimulation increases the sympathetic input to the heart and blood vessels (Folkow & Rubinstein, 1966; Ninomiya, Judy & Wilson, 1970), and in addition, discrete electrical stimulation of the PMH has been shown in this study to elicit cardioacceleration and arterial hypertension due to sympathetic excitation. As the lateral reticular nucleus (LRN) has been shown to be the site of origin of descending fibres which terminate on intermediolateral neurones and electrical stimulation of the LRN produces cardioacceleration and arterial hypertension by sympathetic excitation (Henry & Calaresu, 1974) the demonstration in the present investigation of a descending pathway from the PMH that projects to the LRN prompts the suggestion that the hypothalamic induced changes in sympathetic activity are mediated by the LRN.

As medullary field potentials were also recorded in the dorsal longitudinal fasciculus (DLF) and the hypothalamic sites of stimulation were located in the generally accepted site of origin of the DLF (Crosby Humphrey & Lauer, 1962) it is suggested that the inhibitory effect of PMH stimulation on medullary and reflex vagal bradycardia is mediated by the DLF. In addition, the finding that the majority of

the field potentials recorded in the DLF were located in the rostral area of the medullary region explored prompts the suggestion that the DLF descends in a medial position and gives rise to collateral fibres which project to nuclei in the medulla involved in control of the cardiovascular system. This suggestion is supported by the work of Crosby & Woodburne (1951) who traced the fibre tract and sites of termination of the DLF in the macaque. They found that the DLF descends in a dorsomedial position in the brain stem and contributes fibres to the region of the dorsal nucleus of the vagus, the medial reticular formation and likely the n. ambiguus. If it is accepted that the DLF mediates hypothalamic inhibition of vagal bradycardia, this study provides the first electrophysiclogical evidence of the medullary sites of termination of the DLF.

B. Conclusions

This investigation has contributed to the understanding of central control of the cardiovascular system by providing evidence about the role of suprabulbar structures in the control of cardiovascular reflexes.

In the initial experiments it was found that stimulation of a discrete vasomotor region in the posteromedial hypothalamus (PMH) of cats elicits cardioacceleration and arterial hypertension due to an increase in sympathetic activity. The demonstration that stimulation of baroreceptors and chemoreceptors alters the activity of neurones in the PMH provides the first direct evidence that in

addition to the medulla and pons, suprabulbar structures are involved in the mediation of cardiovascular reflexes. With regard to suprabulbar control of cardiovascular reflexes, it is well documented that the hypothalamus exerts an inhibitory influence on the vagal component of the baroreceptor reflex. However, as there is uncertainty concerning the efferent pathway of the chemoreceptor reflex, it was considered necessa to identify the efferent components of this reflex before investigating the influence of suprabulbar structures on the baroreceptor and chemoreceptor reflexes and it was found that the hypertension elicited by intracarotid injection of sodium cyanide is mediated by sympathetic excitation. The bradycardia elicited by excitation of carotid body chemoreceptors was found to be due exclusively to vagal excitation and to be mediated by both vagus nerves. In addition, the significant increase in the magnitude of the chemoreceptor induced vagal bradycardia after midcollicular decerebration is the first demonstration that the chemoreceptor reflex like the baroreceptor reflex is subject to inhibitory influences from structures rostral to the level of decerebration. One of the sites of origin of this inhibitory influence is the PMH as electrical stimulation of this region was shown to inhibit the reflex vagal bradycardia elicited by selective stimulation of carotid body chemoreceptors; in addition, the PMH was found to inhibit baroreceptor induced vagal bradycardia. This inhibitory influence was shown to be mediated by crossed as well as uncrossed pathways.

After it was shown that the PMH inhibits reflex vagal bradycardia, the site of interaction of this inhibitory effect and the

medullary structures mediating reflex vagal bradycardia were studied. The data obtained by localizing medullary cardioinhibitory sites strongly suggests that the nucleus ambiguus (NA) is the site of origin of cardioinhibitory neurones and that reflex excitation of these cardioinhibitory neurones involves an interneurone located in the nucleus of tractus solitarius (NTS). In addition, the demonstration that electrical stimulation of the PMH at sites that inhibited reflex vagal bradycardia inhibits the bradycardia elicited by stimulation of the NTS and dorsal nucleus medullae oblongatae centralis (dorsal MOC) but not the cardiac slowing elicited by stimulation of the NA provides additional support for the hypothesis that the NA is the site of origin of cardioinhibitory neurones and that the NTS and dorsal MOC are structures involved in the reflex excitation of these neurones. Furthermore, the results provide indirect evidence that the inhibitory effect of hypothalamic stimulation on reflex vagal bradycardia is mediated by medullary structures. The finding that field potentials elicited by stimulation of the PMH were recorded in discrete medullary nuclei which have been shown in the present investigation to mediate reflex vagal bradycardia, and, in previous studies, to inhibit the baroreceptor reflex and to excite spinal sympathetic neurones is the first electrophysiological demonstration that hypothalamic vasomotor areas project to discrete medullary nuclei involved in central and reflex control of the cardiovascular system. In addition, these results strongly support the hypothesis that the cardiovascular changes elicited during hypothalamic stimulation are mediated by altering the activity of discrete vasomotor areas in the medulla.

In view of the demonstration that the PMH receives input from baroreceptors and chemoreceptors, that stimulation of the PMH inhibits reflex vagal bradycardia and medullary bradycardia and that the PMH projects to discrete medullary nuclei involved in central and reflex control of the heart and blood vessels it is concluded that the posteromedial hypothalamus plays an important role in the mediation and control of the baroreceptor and chemoreceptor reflex.

SUMMARY

1. This study was done to localize the neural structures and the mechanism(s) involved in the hypothalamic inhibition of reflex vagal bradycardia.

2. A discrete vasomotor region in the hypothalamus was identified in chloralose anaesthetized cats by studying the effect of hypothalamic stimulation on heart rate and arterial pressure, by recording the response of single hypothalamic neurones to selective stimulation of baroreceptors and chemoreceptors, and by demonstrating the inhibitory effect of stimulation of this region on reflex vagal bradycardia.

3. In nine chloralosed cats, electrical stimulation at 20 histologically confirmed sites in a discrete region of the posteromedial hypothalamus (PMH) elicited cardioacceleration and arterial hypertension. The cardioacceleration was due to sympathetic excitation as the response was not affected by bilateral cervical vagotomy (3 cats) but was abolished in five cats by intravenous administration of propranolol (1.5 mg/kg).

4. In 14 cats, the response of 51 single units in the PMH to electrical stimulation of the carotid sinus nerve (CSN) was recorded with extracellular stainless steel electrodes: 16 of these units were inhibited (latency 68± 9 msec) and seven were excited (latency 29± 3.7 msec). In five of the 16 units that were inhibited by CSN stimulation baroreceptors were selectively stimulated by intravenous administration of noradrenaline (0.5 – 2.0 µg/kg) and were shown to inhibit the

spontaneous activity; selective excitation of carotid body chemoreceptors by close intra-arterial injection of sodium cyanide (50 - 100 μg/kg) attempted in three of these five units did not alter the spontaneous activity. Selective chemoreceptor excitation attempted in one of the seven units excited by CSN stimulation increased the discharge frequency while noradrenaline administration failed to elicit a response. These results suggest that, in addition to the medulla and pons, a vasomotor region in the PMH is also involved in the mediation of baroreceptor and chemoreceptor reflexes.

5. As the efferent pathways mediating chemoreceptor bradycardia are uncertain the identification of these pathways was considered a necessary prerequisite before investigating the effect of hypothalamic stimulation on chemoreceptor induced changes in heart rate. In 29 cats selective stimulation of right carotid body chemoreceptors consistently elicited cardiac slowing and arterial hypertension. The cardiac slowing was due solely to vagal excitation as the magnitude of the bradycardia was not affected by administration of propranolol (9 cats) or by spinal transection at C_7 (10 cats) but was significantly reduced after right cervical vagotomy and abolished after bilateral vagotomy (12 cats). The chemoreceptor induced arterial hypertension was due to sympathetic excitation as the response was abolished by spinal transection at C_7 (10 cats).

6. After midcollicular decerebration in four cats the magnitude of cardiac slowing elicited by chemoreceptor stimulation was significantly increased. These results demonstrated the existence of a tonic inhibitory influence by supra-collicular structures on chemoreceptor induced

vagal bradycardia.

7. In 18 cats transected at C_7 electrical stimulation of 41 histologically confirmed sites in the right and left PMH inhibited the reflex vagal bradycardia elicited by stimulation of the right CSN. In addition, stimulation of 39 sites in the right and left PMH inhibited the vagal bradycardia elicited by selective excitation of baroreceptors and right carotid body chemoreceptors. These results demonstrated that the PMH inhibits reflex vagal bradycardia and that a possible site of origin of the tonic inhibitory influence exerted on chemoreceptor bradycardia by supracollicular structures is the PMH.

8. To identify medullary structures involved in hypothalamic inhibition of reflex vagal bradycardia the medulla was systematically explored for vagal cardioinhibitory sites and the effect of simultaneous stimulation of the PMH on the magnitude of this bradycardia was investigated.

9. The right side of the medulla (1 mm caudal to 4 mm rostral to the obex, 5 mm lateral) was systematically explored in 27 chloralosed cats transected at C_7. Electrical stimulation with unipolar electrodes elicited a vagal bradycardia from 160 histologically confirmed sites which were arbitrarily classified as dorsal or ventral with respect to the intramedullary vagal rootlets. The majority of the dorsal sites (41/51) were located in the n. of tractus solitarius (NTS) and in the dorsal n. medullae oblongatae centralis (MOC), and the majority of the ventral sites (86/97) were located in the n. ambiguus (NA) and in the ventral MOC; in addition, 12 sites were found between the ventral MOC and the hypoglossal n. and were classified as dorsomedial. The

responses from the dorsal, ventral and dorsomedial sites were not significantly different in magnitude. After ipsilateral vagotomy stimulation of the NA (11 cats), the NTS (4 cats) and the ventral MOC (3 cats) elicited an increase in heart rate.

10. In six experiments administration of sodium pentobarbital (10 mg/kg i.v.) reduced the magnitude of the bradycardia elicited from the NTS significantly more than the bradycardia from the NA and from the peripheral vagus. These results support the possibility that cardioinhibitory neurones are located in the region of the NA and that reflex excitation of these neurones involves an interneurone located in the NTS.

11. In nine chloralosed cats transected at C_7, electrical stimulation of 10 sites in the right PMH and six sites in the left PMH that inhibited reflex vagal bradycardia induced by noradrenaline administration was found to inhibit the vagal bradycardia elicited by stimulation of 12 sites in the right medulla, seven in the NTS and five in the dorsal MOC. However, the bradycardia elicited by stimulation of 11 sites in the right NA was not affected by PMH stimulation.

12. To investigate the sites of termination of inhibitory hypothalamo-medullary pathways field potentials were recorded in the medulla during electrical stimulation of the PMH.

13. In 21 chloralosed cats transected at C_7, field potentials were recorded at 405 sites in the right and left medulla during stimulation of the ipsilateral and contralateral PMH. The field potentials were located in the inferior olivary n., lateral reticular n., NTS, ventral and dorsal MOC, NA, parahypoglossal area, and dorsal

longitudinal fasciculus. The field potentials had a peak latency of 19-53 msec and did not follow frequencies of stimulation greater than 26 Hz, were not affected by muscle paralysis (gallamine triethiode, 5 mg/kg i.v.) but were abolished by barbiturate (sodium pentobarbital, 20 mg/kg i.v.) and by asphyxia. Ipsilateral medullary field potentials were abolished by ipsilateral hemitransection at the ponto-medullary junction in six cats (3 in the right and 3 in the left medulla). However, ipsilateral transection only reduced the amplitude of one contralateral field potentials in the right medulla and three contralateral field potentials in the left medulla and bilateral transection was required to abolish these contralateral responses.

14. It is concluded that the inhibitory effect of PMH stimulation on reflex vagal bradycardia is mediated by crossed and uncrossed pathways which alter the electrical activity of neurones located in discrete vasomotor structures in the medulla. In addition, these data suggest that the dorsal longitudinal fasciculus is the descending pathway mediating the cardiovascular responses and inhibitory effects observed during stimulation of the hypothalamus.

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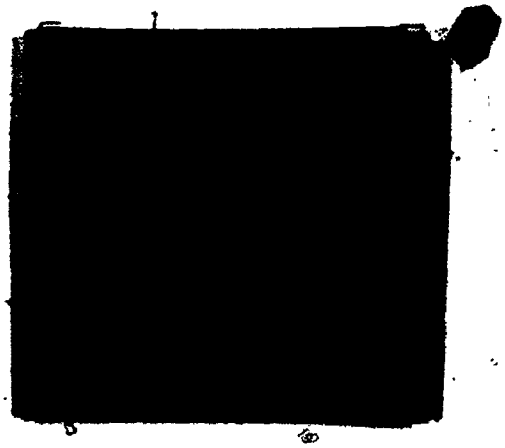

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