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CARDIOVASCULAR REGULATION BY THE PARAVENTRICULAR NUCLEUS

BY

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Cardiovascular Regulation by the Paraventricular Nucleus" submitted by Bonnie Bagdan in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

The paraventricular nucleus (PVN) of the hypothalamus projects both to the posterior pituitary (PP) and to sites within the central nervous system which are important for cardiovascular regulation. Experiments were undertaken to examine responses of centrally projecting PVN neurons to a variety of stimuli previously reported to alter activity of PVN neurons which project to the PP (PVN-PP cells).

Rats were anesthetized with halothane and their femoral artery and vein cannulated for blood pressure measurement and drug administration, respectively. Bipolar stimulating electrodes were placed in the PP, as well as in the ventrolateral medulla (VLM) and nucleus tractus solitarius (NTS). Antidromic potentials were recorded extracellularly in the PVN. The activity of antidromically identified PVN neurons were observed in response to blood pressure changes as well as other stimuli. A total of 176 antidromically activated neurons were found, 58 by PP stimulation only, 46 followed NTS stimulation only (PVN-NTS cells) and 68 followed VLM stimulation only (PVN-VLM cells). Three neurons were antidromically activated following stimulation of both the PP and the brain stem.

The typical response of PVN-PP cell activity to increased or decreased blood pressure was a reciprocal response, with increased cell firing when blood pressure decreased but reduced cell firing when blood pressure was raised. These neurons also were typically excited by such stimuli as hemorrhage, increased plasma osmolality, and systemic injections of cholecystokinin octapeptide (CCK). In contrast,

however, most of the brain stem projecting PVN neurons were unresponsive to these stimuli. The few neurons that did respond to changes in blood pressure were all inhibited by either an increase or decrease of blood pressure. None of the other stimuli were found to be effective to modulate the activity of these neurons. Although from this data, it seems that the cells projecting to different areas do not act in the same manner to specific stimuli. However, the three PVN neurons which projected to both the PP and VLM or NTS, suggest that there could be coordinated activation of the two pathways.

The finding of a few neurons in the PVN that project to the NTS or VLM which respond to alterations in blood pressure, osmolality, or volume suggest that such neurons may share with the PVN-PP neurons a role in cardiovascular control by the PVN. However, the lack of responses of the majority of centrally projecting PVN neurons suggest roles for such neurons in other activities as well.

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LIST OF ABBREVIATIONS

AG	Angiotensin II
AV3V	Anterior wall of the third ventricle
AVP	Arginine vasopressin
BSA	Bovine serum albumin
° C	Degrees centigrade
CCK	Cholecystokinin
CNS	Central nervous system
DVC	Dorsal vagal complex
g	gram
hem	hemorrhage
Hz	Hertz
IML	Intermediolateral column of the spinal cord
icv	Intracerebroventricular
im	Intramuscular
ip	Intraperitoneal
iv	Intravenous
kg	Kilogram
MNC	Magnocellular neuron
Mx	Methoxamine
MOhm	Mega-Ohm
M	Molar

mg	Milligram
min	Minute
ml	Milliliter
mm	Millimeter
mmHg	Millimeters of mercury
mOsm	Milliosmole
msec	Millisecond
mV	Millivolts
NP	Nitroprusside
ng	Nanogram
NTS	Nucleus Tractus Solitarius
OT	Oxytocin
PVN	Paraventricular nucleus of the hypothalamus
pO_2	Partial pressure of oxygen
pCO_2	Partial pressure of carbon dioxide
PP	Posterior Pituitary
s	Second
SFO	Subfornical Organ
SON	Supraoptic nucleus of the hypothalamus
µg	Microgram
µl	Microliter
V	Volt

I. INTRODUCTION

A. REVIEW OF CARDIOVASCULAR REGULATION

The circulatory, endocrine and nervous systems constitute the principle coordinating and integrating systems of the body. The circulatory system serves to transport and distribute essential substances to the tissues and remove by-products of metabolism. Regulation of the cardiovascular system involves the activation of several groups of receptors whose inputs to the central nervous system (CNS) result in negative feedback loops. The variables controlled by such negative feedback include arterial blood pressure, cardiac output, circulating blood volume, and arterial blood gas tensions. The levels of these variables are monitored by various peripheral receptors whose afferent activity to the CNS alters autonomic outflow to the cardiovascular system and release of humoral substances to produce appropriate physiological responses.

Predominant reflex control of circulation is mediated by receptors located within the cardiovascular system. These receptors fall into two major categories: mechanoreceptors and chemoreceptors.

(1) Mechanoreceptors - mechanoreceptors are situated in main systemic vessels as well as the heart. Two types of mechanoreceptors are identified: baroreceptors and cardiac or atrial receptors.

A. Baroreceptors are localized in the aortic arch and carotid sinuses and their function is to monitor arterial blood pressure. Each receptor has a

threshold for activation in the range of 30-150 mmHg (reviewed in Loewy and Spyer,1990). Receptors located in the aortic arch relay information to the CNS via a branch of the vagus known as the aortic depressor nerve. Receptors in the carotid sinus relay baroreceptor information through a branch of the glossopharyngeal nerve known as the carotid sinus nerve.

With increases in blood pressure, the frequency of baroreceptor activity is increased, whereas the converse is true with a reduction in blood pressure. Activation of these baroreceptors increases electrical activity of neurons in the NTS through excitatory synapses. The NTS, in turn, sends inhibitory projections to other nuclei within the CNS. This results in decreased activity of preganglionic sympathetic nerves to peripheral blood vessels and heart, peripheral vasodilation and a reduction of blood pressure. Contributing to this reduction of blood pressure is bradycardia resulting from stimulation of the vagal cardioinhibitory neurons arising largely in the nucleus ambiguus and to a lesser extent in the dorsal motor nucleus of the vagus (Berne and Levy, 1985). Thus, arterial blood pressure falls due to the decline in cardiac output and peripheral resistance. In contrast, decreased arterial pressure is compensated for by a decrease in baroreceptor firing, resulting in activation of the neurohypophyseal and sympathetic nervous systems.

Vascular resistance is mainly provided by the state of constriction of arterioles, which are principal points of resistance to blood flow in the circulatory system. Adjustment in the degree of contraction of the smooth muscle of these vessels allows regulation of tissue blood flow and aids in arterial blood pressure control. Control

of these resistance vessels is twofold: neurohormonally by arginine vasopressin (AVP) and other hormones, and via autonomic outflow. Sympathetic activation or release of hormones such as AVP into the bloodstream enhances vascular resistance by constricting arterioles. This will be discussed in further detail later.

The baroreflex operates continuously to provide short-term adjustments to arterial blood pressure as well as heart rate. Thus, baroreceptor inputs are crucial for maintaining cardiac output.

B. Cardiac or atrial receptors have endings located either in great veins near their entry into the heart, or within walls of the atria themselves. These receptors are low pressure receptors which perceive the volume of venous return and relay this information regarding circulating blood volume through the vagus nerve to the CNS. Thus, atrial receptors play an important role in monitoring venous return and providing the CNS with information related to atrial distention or contraction. Increased activity of these receptors due to atrial distention involves a sympathetically mediated tachycardia as well as decreased sympathetic discharge to resistance vessels and the kidney, indicating a putative role in maintenance of vascular fluid volume. Generalized arteriolar vasoconstriction is a salient component of the reflex response to haemorrhage. Atrial receptors also play a role in regulation of vasomotor tone and urine output by stimulation or inhibition of angiotensin or AVP.

Thus, both the atrial and baroreceptors are necessary for full expression of blood pressure regulation.

(2) Chemoreceptors - The chief set of receptors with chemoreceptor functions

are the arterial chemoreceptors found within carotid bodies and the aortic arch. These receptors relay information about the chemical balance of the blood (pH, PO_2, PCO_2) to the sinus and aortic nerves, respectively. Chemoreceptors are primarily concerned with regulation of respiration. However, they do reflexly influence vasomotor regions to a minor degree.

The afferents of baro-, atrial, and chemoreceptors terminate in the nucleus tractus solitarius of the medulla. From here, several mono- and multisynaptic pathways traverse to magnocellular hypothalamic nuclei, the supraoptic (SON) and paraventricular (PVN) nuclei, as well as other CNS sites. These pathways from the NTS integrate the information necessary to control AVP release which accompanies changes in sympathetic drive to certain vascular beds and heart.

Central nuclei which provide synaptic inputs to AVP neurons seem to serve 2 main functions: regulation of water balance and the cardiovascular system. Structures near the anterior wall of the third ventricle (AV3V), such as the organum vasculosum, and the subfornical organ (SFO) are circumventricular structures of the brain and thus lack a normal blood brain barrier (Dellman and Simpson,1979). These sites, especially the SFO, are specialized for control of fluid balance. Osmosensitive elements of the AV3V and SFO are excited when plasma osmotic pressure rises. These osmotic sensors of the AV3V region send axons to areas within the hypothalamus such as the SON and PVN (Donevan and Ferguson,1988) to stimulate release of humoral factors or neural pathways from these nuclei. In this manner, plasma osmolality is regulated via reflex mechanisms as is blood pressure

and volume. In addition to this, large numbers of angiotensin II (AG) receptors have been localized within the SFO (Mendelsohn et al.,1984). It is well known that conditions which deplete body fluids, such as haemorrhage and dehydration, are accompanied by a rise in circulating levels of AVP. There is no evidence that AG crosses the blood-brain barrier to gain direct access to the PVN, and it has therefore been proposed that circulating AG may act upon cells in circumventricular like the SFO which lack one. In summary, it seems likely that circulating AG influences AVP release by acting on tissue in or near circumventricular organs that lack a blood-brain barrier to peptides. This finding implicates AG in regulation of plasma osmolality via the SFO and its connections.

This information shows that regulation of the cardiovascular system is a complex process involving several areas in the periphery as well as the CNS. Thus, it seem likely that certain areas within the body are required to monitor and integrate all of the physiological processes and this system in balance. The hypothalamic paraventricular nucleus (PVN) has been implicated in such a role because it has been shown to receive information from, and integrate several neuroendocrine, homeostatic & autonomic functions (review by Swanson & Sawchenko,1980 and 1983; Swanson et al.1986) including cardiovascular regulation. The involvement of the PVN in cardiovascular regulation is the focus of this thesis.

B. THE PARAVENTRICULAR NUCLEUS (PVN)

1. Contents of the PVN

The PVN is composed of a heterogeneous population of neurons in the hypothalamus that has been subdivided on the basis of peptide localization, projection sites, location in the nucleus and cell size (Armstrong et al.,1980; Hosoya and Matsushita,1979; McKellar and Loewy, 1981; Swanson and Kuypers, 1980; Swanson et al., 1981; Swanson & Sawchenko'83; Swanson et al. 1986; Swanson et al.1987).

a. Cell Types in the PVN Two general classes of cells are found in the PVN.

(1) The most predominant cell type in the PVN are magnocellular neurons (MNC's) which release the peptides oxytocin (OT) and AVP into the general circulation at the level of the posterior pituitary (PP). Magnocellular somata have a mean diameter of 25.4μm (Sofroniew,1985). As a general rule, magnocellular perikarya project to the PP but not to the brain stem or spinal cord. They have two or three simple dendrites which stay, for the most part, within morphological boundaries of the PVN (Armstrong et al.,1980; Swanson and Sawchenko,1983).

(2) The second type of cells are the parvocellular (small) neurons which secrete hormones into the hypophyseal portal system as well as innervate several areas within the CNS (Rho & Swanson,1988) including areas as distant as the spinal cord (Swanson and Kuypers,1980; Cechetto and Saper,1988). These cells are smaller than the MNC's, having a mean diameter of 16.6 - 19.8μm (Sofroniew 1985). The dendrites and accompanying spines of parvocellular neurons are remarkably similar

to those of MNC's, however, parvocellular cells have more elaborate dendritic trees than do MNC's (Rho and Swanson, 1988). This observation may be important for the function of MNC's versus parvocellular PVN neurons, and will be addressed further in the discussion. Parvocellular neurons of the PVN appear to have biosynthetic or storage features which differ from MNC's, because they stain immunohistochemically only if the animal was pretreated with colchicine, whereas MNC's stain even in the absence of colchicine (Cechetto and Saper,1988).

b. Subdivisions of the PVN

In the rat, the PVN occupies less than 1/3 of a millimeter cubed of tissue on either side of the third ventricle, however it contains some ten thousand neurons and can be divided into at least 8 clearly distinguishable subdivisions which contain several biologically active substances (Swanson & Sawchenko,1983). However, for the purposes of this thesis, a less stringent method of subdividing this nucleus based on Armstong et al. (1980) is shown. This involves three main functional regions of the PVN :

(1) A group of magnocellular neurosecretory neurons which synthesize OT & AVP, as well as other peptides, and send their axons to the PP. These peptide hormones are then released into the general circulation upon appropriate stimulation.

(2) A group of medial & periventricular parvocellular neurosecretory neurons that produce pituitary releasing factors, such as corticotropin-releasing factor and thyrotropin-releasing factor, and send their axons to the median eminence. Here

they terminate in close proximity to capillaries, are released, and transported by the hypothalamo-hypophyseal portal system to the anterior pituitary where they influence the release of anterior pituitary hormones

(3) A collection of parvocellular neurons that lies dorsal, lateral and ventral to magnocellular neurons which has widespread connections within the CNS. Neurons of this group project to autonomic centers of the brain stem and spinal cord.

As a general rule, these nuclear subdivisions have distinct projection targets, with magnocellular neurons projecting to the PP and parvocellular neurons projecting to areas of the CNS such as the brain stem and spinal cord (Rho and Swanson,1988; Swanson and Kuypers,1980) although some zones of overlap do occur (Armstrong et al.,1980). In fact, Hosoya and Matsushita (1979) found that a very small number of PVN cells projected both to the PP and to the brain stem. Thus, although cells projecting to the brain stem and spinal cord differ morphologically from those projecting to the PP (Hosoya and Matsushita,1979), it is not possible to predict the appearance of cells that might project to both terminal fields.

2. Electrophysiology of PVN Neurons

As well as being differentiated morphologically and by their location within the nucleus, magnocellular and parvocellular neurons of the PVN can be differentiated by their electrophysiological properties. Magnocellular neurons have resting membrane potentials ranging from -75 to -55 mV, and spike threshold near -60mV (Bourque,1987). Action potentials of MNC's are carried by low threshold

sodium potentials and high threshold calcium currents (Bourque and Renaud,1985). Parvocellular cells display low-threshold action potentials that generate 1 - 2 action potentials, which differs from the MNC's which lack low-threshold potentials.

Magnocellular vasopressinergic neurons have been found to display a unique firing pattern known as phasic activity (Bourque,1987). Phasic activity consists of periods of fairly steady discharge (5-20 spikes/s) lasting several seconds or even minutes, interspersed with periods of complete silence or a few intermittent action potentials. The duration of both the active and quiescent phases is variable, both for individual neurons and between neurons. It seems likely that phasic activity is an intrinsic property of the neurons themselves because it can be recorded from neurons in hypothalamic slices which are devoid of many other afferent inputs, suggesting that major input from remote sites is not essential. Also, Gahwiler and Dreifuss (1979) found that phasic bursts occur by actions of pacemaker cells, whose activity was a function of membrane potential but not synaptic in origin, and follower cells whose activity was synaptic in origin and were unaffected by current injection. The switching on and off during phasic activity is a property of the individual neuron, but one which would be profoundly influenced by its neighbours. This interaction could be further enhanced by increasing the degree of neuron-to-neuron apposition by changes in ultrastructure of glial elements which is a process reported to occur during dehydration and other stimuli (Hatton and Tweedle,1982). One explanation for the genesis of phasic activity is that it represents the build up and decay of some material, possibly even of simple ions such as potassium or calcium ions.

Thus, the two types of neurons in the PVN can be differentiated by their electrophysiological properties as well as by their anatomical distinctions.

3. PVN and Cardiovascular Regulation

Support for the role of the PVN in regulating the cardiovascular system comes from several areas. The afferent and efferent connections of this nucleus, its contents, and the effect of stimulation of this nucleus implicate it in cardiovascular regulation.

a. Magnocellular PVN Neurons

i. Afferents

Central nuclei that provide synaptic inputs to magnocellular neurons of the PVN appear to serve two main functions: regulation of water balance and the cardiovascular system. Inputs to the PVN have been studied in rats with a variety of neuroanatomical methods. The PVN receives direct inputs from several nuclei within the CNS. These include brain stem areas such as the ventrolateral medulla (VLM) (Kannan et al.,1984; Ciriello and Caverson,1984), the nucleus tractus solitarius (NTS) and dorsal nucleus of the vagus (DMV) (Ricardo and Koh,1978), parabrachial nucleus, laterodorsal tegmental nucleus and the locus coeruleus (Tribollet & Dreifuss'81). The PVN also receives projections from hypothalamic areas including various parts of the preoptic area (Swanson 1976), the ventromedial nucleus (Saper et al.,1976), the anterior hypothalamic area (Saper et al.,1978), lateral

hypothalamic area (Saper et al.,1979), the suprachiasmatic nucleus (Swanson and Cowan,1975; Tribollet and Dreifuss,1981) and the arcuate nucleus of the hypothalamus (Sawchenko et al.,1982). Higher forebrain structures of the telencephalon, such as the lateral septum, medial nucleus of the amygdala, and hippocampal formation, innervate cells of the PVN (Tribollet & Dreifuss,1981). Other areas of the brain which project to the PVN include the SFO (Tribollet & Dreifuss,1981; Gutman et al.,1985; Donevan and Ferguson,1988), bed nucleus of the stria terminalis (Donevan and Ferguson,1988), and the raphe nuclei (Tribollet and Dreifuss,1981). Each input to the PVN has a unique distribution within the nucleus, and when all of the inputs to this nucleus are considered in relation to the organization of its projection neurons, it seems clear that the circuitry within the nucleus is highly differentiated (Swanson and Sawchenko,1983).

The majority of projections to the PVN innervate parvocellular neurons of the PVN, however areas with prominent projections to the magnocellular neurons of the PVN include the VLM, dorsomedial nucleus and median preoptic nucleus of the hypothalamus. The SFO and arcuate nucleus project heavily to most parts of PVN, including magnocellular neurons.

Neurotransmitters - The neurotransmitters used by these projections to synapse on PVN neurons are not known for certain, however noradrenaline is a chief putative excitatory neurotransmitter of AVP magnocellular neurons. Studies indicate that three cell groups provide almost all of the noradrenergic and adrenergic fibers to the hypothalamus. These include the A1 cell group of the VLM, the A2 cell

group of the NTS, and the A6 cell group of the locus coeruleus (Tribollet and Dreifuss,1981). However, the A1 cell group appears to be the main noradrenergic cell group that has a direct effect on AVP neurons. Adrenergic input has also been found to be involved with innervation of the PVN, shown by the C1 group of the VLM (reviewed in Loewy and Spyer,1990). In addition, immunoreactive neuropeptide Y has been found on both the A1 noradrenaline and C1 adrenergic neurons that project to magnocellular nuclei (Blessing et al.,1986). Rather than being a major excitatory transmitter of A1 or C1 cells that project to these magnocellular neurons, neuropeptide Y probably acts with noradrenaline to potentiate AVP release. There is also ACTH input to magnocellular neurons of the PVN from arcuate nucleus projection, and beta-endorphin has been found to be co-localized in these neurons as well (Sawchenko et al.,1982).

Responses to Afferent Information - Various stimuli terminating on PVN magnocellular neurons modulate the activity of these cells. Stimuli shown to selectively activate AVP into the peripheral circulation include stimulation of renal nerve afferents (Hasser et al.,1987), nociceptive somatic stimuli (Day and Sibbald,1990) and blood pressure changes (Renaud et al.,1987). Changes in blood pressure are sensed by baroreceptors and the information travels through the NTS and VLM as previously mentioned. Both the NTS and VLM have been found to project to the PVN, and thus this is one mechanism whereby the PVN receives such afferent information and is able to respond to it.

Other stimuli selectively activate OT-secreting magnocellular PVN neurons,

such as suckling (Wakerley and Lincoln,1973) gastric distention and the response to systemic administration of cholecystokinin (CCK), lithium chloride and copper sulfate (Verbalis et al.,1986; Renaud et al.,1987). Gastric distention and CCK administration have both been found to stimulate the vagus, which results in activation of OT neurons within the PVN.

Finally, certain stimuli have been shown to activate both OT and AVP secreting PVN magnocellular neurons. These stimuli include hypovolemia and haemorrhage (Weitzman, 1978), hyperosmolality (Wakerley et al.,1978), and circulating angiotensin II (AG)(Ferguson and Renaud,1986). As previously discussed, changes in blood volume as during haemorrhage are sensed by atrial receptors, whose neural output is sent to brain stem nuclei such as the NTS and VLM. From here, this information about blood volume is sent to the PVN, just as blood pressure manipulations are. Information about plasma osmolality changes as well as AG in the circulation are detected by the SFO which projects to the PVN. Thus, it seems that the PVN is in an ideal position to integrate inputs regarding all aspects of the cardiovascular system.

ii. Efferent Projections

The neurohypophyseal hormones AVP and OT are produced by separate hypothalamic magnocellular neurons (Vandesande and Dierickz,1975), most of which are concentrated in two distinct cell groups of the hypothalamus, the PVN and SON. Unlike the SON which is a homogeneous population of AVP and OT cells which

project solely to the PP, the PVN is a relatively heterogeneous population of parvocellular and magnocellular neurons which contain a multitude of biologically active substances. The PVN also differs from the SON in that it sends neuronal projections to several areas besides the PP, including numerous extrahypothalamic areas within the CNS. Neurons which project from the PVN to the PP form the hypothalamo-neurohypophyseal tract which axonally transports peptide hormones OT and AVP, along with a number of other colocalized peptides, from the magnocellular neurosecretory cells to the PP. Here, the hormones are released into the general circulation when activated by appropriate stimuli which were discussed previously.

Release of OT into the bloodstream results in contraction of uterine and mammary smooth muscle, actions which are important for lactation and parturition. The release of OT from the PP into the circulation may also have a function in inhibition of food intake and gastric emptying (McCann et al.,1989). This action of OT may occur by this peptide acting on vascular smooth muscle in the mesenteric arteries, as does AVP (Altura and Altura,1977), to decrease blood flow to the intestines and thus limiting absorption of ingested materials.

Circulating AVP activates several areas involved in cardiovascular homeostasis by binding to specialized receptors on the cell membrane. There are 2 general classes of AVP receptors (Harris and Loewy,1987).

(1). One group, the V_2 receptors, are located largely on cell membranes of renal tubules. Binding of AVP to V_2 AVP receptors leads to increased permeability of basement membrane to water, brought about by activation of adenylate cyclase

second messenger system. This results in increased water uptake from the distal convoluted tubule and collecting duct of nephrons.

(2). The second group of AVP receptors (V_1) of relevance to the cardiovascular system is located on the vascular smooth muscle cell membrane. Binding of AVP to V_1 receptors leads to activation of the phosphatidylinositol second messenger system and result in contraction of the muscle, thus increasing blood pressure.

Thus, AVP released from the hypothalamo-neurohypophyseal tract helps to regulate the cardiovascular system by 2 distinct physiological mechanisms. It acts indirectly via V_2 receptors in the kidney to cause water retention, expansion of extracellular fluid volume, increased venous return, and increased cardiac output and blood pressure. It also acts directly to cause vasocontriction of blood vessels (V_1 receptors) and thus increases blood pressure.

b. Parvocellular PVN Neurons

i. Afferents

Most of the brain areas listed under magnocellular afferent projections above also project to parvocellular neurons of the PVN. For example, most PVN projections from the NTS and DVC innervate parvocellular neurons (Ricardo and Koh,1978), some cells from the VLM project to these smaller neurons (Swanson and Sawchenko,1982), and the SFO and arcuate nucleus project to the parvocellular PVN as well. Most of the neuronal projections from the hypothalamus to the PVN listed

above terminate on parvocellular neurons except for projections from the dorsomedial and preoptic hypothalamic nuclei. In addition, the parabrachial nucleus projects preferentially to parvocellular PVN neurons (Tribollet and Dreifuss,1981; Saper and Loewy,1980) as does the suprachiasmatic nucleus (Berk and Finkelstein,1981). There are also serotonergic inputs to PVN from both the raphe nuclei (Tribollet and Dreifuss,1981) and some areas of the VLM (Loewy et al.,1981). Finally, the arcuate nucleus has been shown to project to parvocellular OT PVN neurons as well as to magnocellular cells as described above (Tribollet & Dreifuss,1981).

Neurotransmitters - A multitude of different putative neurotransmitters have been identified from these afferent projections. These include noradrenaline from VLM and NTS projections, serotonin from the raphe nuclei and some VLM neurons, ACTH and possibly beta-endorphin by arcuate nuclear projections (Tribollet & Dreifuss,1981), and vasointestinal peptide from the suprachiasmatic nucleus neurons (Swanson & Sawchenko,1980). The biochemical specificity of parabrachial input is not known, but is possibly enkephalin or neurotensin (Tribollet & Dreifuss,1981).

Responses to Afferent Information - The function of several of these projections is unknown. Some putative functions include control of urination by the laterodorsal tegmental nuclear projections (Tribollet & Dreifuss,1981), mediation of

circadian rhythms in neuroendocrine and autonomic functions influenced by PVN via suprachiasmatic projections, and the regulation of anterior pituitary function via its projections from the arcuate nucleus to the median eminance (Tribollet and Dreifuss,1981). The parvocellular PVN has also been implicated in the regulation of cardiovascular function due to its innervation by areas known to be involved in cardiovascular reflexes such as the VLM and NTS (Saper et al.,1976; Silverman et al.,1985; Swanson,1977) as well as other projections to areas known to be involved in cardiovascular control.

ii. Efferent Projections

Morphological studies in recent years have shown that PVN axons containing the classic neurohypophyseal peptides, AVP and OT, are found not only within the hypothalamo-neurohypophyseal tract but also in a number of projections to the CNS including the locus coeruleus, NTS, DVC, intermediolateral cell column of the spinal cord (IML), and VLM (Buijs,1978; Sofroniew,1980; Swanson and Kuypers,1980; Sofroniew and Schrell,1981; Zerihun and Harris,1983; Kannan and Yamashita,1983; Ciriello and Calaresu,1980; Swanson,1977; Yamashita et al.,1984; Ruggiero et al.,1984). These areas are known to be involved in the regulation of cardiovascular function (Nilaver et al.,1980) and contain AVP receptors resembling the V_1 subtype (Jard et al.,1987; Raggenbass et al.,1987). However, in addition, these PVN neurons have also been shown to contain somatostatin (Sawchenko and Swanson,1982; Fisher et al.,1979), dopamine (Swanson et al.,1981), opiate peptides

such as enkephalin (Sawchenko and Swanson,1982), substance P (Ljungdahl et al.,1978), neurotensin (Kahn et al.,1980) as well as several others.

Function of Central PVN Efferents.

Parvocellular PVN neurons have not been studied as extensively as magnocellular neurons of the PVN. As a consequence, little is known about their function and neurotransmitters, as shown above. One method to determine the function of the parvocellular PVN neurons is to stimulate their cell bodies. For example, electrical or chemical PVN stimulation evokes an increase in mean arterial pressure (Ciriello and Calaresu,1980; Kannan et al.,1989; Pittman and Franklin,1985) which was at least partly due to the central release of AVP (Cowley and Liard,1985; Ciriello and Calaresu,1980). Stimulation of the PVN also increases the concentration of AVP and OT in several CNS areas known to receive PVN vasopressinergic input such as the NTS,VLM, and spinal cord (Pittman et al.,1984; Matsuguchi et al.,1982; Landgraf et al.,1990) as well as result in an increase of sympathetic outflow (Yamashita et al.,1984; Pittman et al.,1984; Kannan et al.,1989; Pittman and Franklin,1985). These cardiovascular responses evoked by PVN stimulation support the hypothesis that the PVN is involved in the central regulation of the cardiovascular system.

Another useful method to determine the function of the centrally projecting PVN neurons is to inject putative neurotransmitters into the central sites which receive PVN projections. For example, microinjection of AVP

intracerebroventricularly (icv) (Pittman et al.,1982a; Martin et al.,1985) or into various sites known to receive PVN innervation results in a pressor response consisting of increased systolic and diastolic blood pressure as well as increased heart rate. For example, Rohmeiss et al. (1986) found that icv AVP injection causes a dose dependent increase in mean arterial pressure, heart rate and sympathetic nerve activity. These pressor responses were all blocked by icv pretreatment with the V_1 receptor antagonist $d(CH_2)_5Tyr(Me)AVP$ and inhibited by peripheral alpha-adrenoreceptor or ganglionic blockade, although intravenous injection of the V_1 antagonist does not alter the pressor response (Rohmeiss et al.,1986). Similar pressor responses were also elicited when AVP is microinjected into the locus coeruleus (Berecek et al.,1984), NTS (Matsuguchi et al.,1982: Martin et al.,1985), VLM (Dampney et al.,1985) and spinal cord (Tan and Tsou,1986; Riphagen and Pittman,1989). These studies suggest a central action of AVP in cardiovascular regulation in areas in which the PVN projections terminate. Similar studies using other peptides present in PVN centrally projecting neurons, such as corticotropin releasing factor (CRF), have been performed with comparable results (Fisher et al.,1983).

Mechanism of Action - A possible mechanism of action for centrally projecting PVN neurons in the regulation of the cardiovascular system is to alter sympathetic outflow to the heart and vasculature. In fact, Rohmeiss et al. (1986) postulated that central AVP acts to increase sympathetic stimulation because alpha-

adrenoreceptor (major neural control of peripheral vessels) blockade attenuated the pressor response to central AVP administration. Furthermore, intrathecally injected AVP at spinal levels induces hypertension due to increased sympathetic nerve activity (Tan and Tsou,1986). Thus, centrally acting neurotransmitters may relay information to sympathetic preganglionic neurons within the IML, the origin of sympathetic nervous system motor outflow (Loewy and Spyer,1990). Stimulation of these preganglionic neurons would lead to norepinephrine release at terminal branches on vessels and elicit constriction (alpha adrenergic effect) of the resistance vessels. In this way, centrally projecting PVN neurons could regulate cardiovascular function.

In fact, several interconnected brain stem networks regulate sympathetic outflow via the IML. Reciprocal pathways exist between the NTS, VLM, parabrachial nucleus, and several forebrain areas including the PVN, bed nucleus of the stria terminalis and central nucleus of the amygdala (reviewed by Loewy and Spyer,1990). These reciprocal connections which regulate sympathetic outflow provide the anatomical substrate for cardiovascular regulation. Thus the VLM and NTS seem to be highly involved in cardiovascular regulation, having reciprocal connections with each other (Ross et al.,1985) and each with the PVN.

(1) NTS - The NTS is an important nucleus for cardiovascular regulation by the PVN due to its reciprocal projections with this hypothalamic nuclei and its role as the major relay nucleus for information arising from baro- and cardioreceptor afferents, being the nucleus which receives baroreceptor impulses. In fact, Kannan

and Yamashita (1985) observed that approximately 1/4 of the NTS neurons which receive PVN projections also receive cardiovascular information from baroreceptors. In addition to this evidence, lesions of the NTS have been shown to result in neurogenic hypertension (Doba and Reis,1973). Thus the NTS has a tonic inhibitory control over the cardiovascular system which is modulated by baroreceptor input and PVN innervation.

(2) VLM - The rostral VLM has reciprocal connections with the PVN. Baroreceptor activity strongly inhibits VLM neurons, an action mediated transynaptically via the NTS. The rostral VLM is also a major source of excitatory input to sympathetic preganglionic neurons in the IML of the spinal cord (Loewy et al.,1981; Barman and Gebber,1985). It has been demonstrated electrophysiologically that stimulation of descending PVN neurons excite VLM neurons which subsequently project to the IML (Caverson et al.,1983).

The rostral VLM has been identified as a vasoconstrictor region which is tonically active. Enhanced activity of this nucleus via reflexes, humoral stimuli, or artificial stimulation increases the frequency of impulses reaching terminal branches of sympathetic nerves to the vessels, resulting in increased arterial pressure (Dampney et al.,1982). In contrast, lesions of the VLM cause a large fall in resting arterial pressure (Guertzenstein and Silver,1974). In this manner neural regulation of the peripheral circulation is accomplished primarily by alteration of the neuronal activity in VLM neurons projecting to the IML where preganglionic sympathetic

neurons are located. The PVN is able to modulate the tonic activity from the VLM to the IML through its projections to the VLM.

Neurotransmitters Involved - The issue of the neurotransmitter or neuromodulator that would regulate cardiovascular function when released at PVN projection terminals is frustrating. Vasopressin seems to be the ideal candidate due to the presence of AVP receptors in areas targeted by PVN projections, its pressor action within the CNS, and because of its cardiovascular actions in the periphery. However, Sofroniew and Schrell (1981) found that OT or AVP neurons from the PVN accounted for less than 10% of all PVN neurons projecting to the medulla. In fact, more than 30 putative neurotransmitters have been identified either in cell bodies or presumed terminals of the PVN (Swanson and Sawchenko,1983), including CRF, dopamine, somatostatin, thyrotropin releasing hormone, enkephalin, AG, and neurotensin (Swanson and Kuypers,1980). The role of these substances have not yet been verified, but they could be involved in other known activities of the PVN.

4. Roles of the PVN - Due to the wide variety of neuronal and vascular projections from the PVN, as well as the identification of more than 30 substances contained within this nucleus, the PVN is implicated in a wide variety of functions. It has been suggested in such roles as respiration (McAllen,1987), parturition and lactation (Wakerley and Lincoln,1973), nociception and anaesthesia (Yirmiya et al.,1990; Lang et al.,1983), gastric function (McCann et al.,1989; Leibowitz,1990; Orosco,1990;

Olson,1991), and the generation of circadian rhythms (Tribollet and Dreifuss,1981). Thus, although the PVN may be involved in regulation of the cardiovascular system, it seems to be important for several other functions as well.

5. Integration of Neurohypophyseal and Central Projections of the PVN

For maintenance of homeostasis, neural and humoral mechanisms must cooperate. To understand control of cardiovascular function, the study of interactions between the endocrine and the autonomic nervous system becomes important. Yamashita et al. (1984) emphasized the view that the neuroendocrine system is intimately associated with the autonomic system. For example, neurohypophyseal projections from the PVN are known to regulate cardiovascular function via their release of AVP as a hormone into the peripheral circulation. In addition, Swanson and McKellar (1979) found that the PVN directly, as well as indirectly via the NTS and VLM, innervates specific groups of sympathetic or parasympathetic preganglionic neurons in the spinal cord. These neurons located within the spinal cord regulate the vasodilation of resistance vessels and cardiac function. Thus, the hypothalamus regulates the circulatory system by this route as well. This suggests that the outputs of the magnocellular neurosecretory system and autonomic nervous system are highly integrated at the level of the PVN to regulate cardiovascular function. Electrophysiological findings by Pittman et al. (1981) strengthen the possibility that the central and peripheral PVN projections act in a coordinated manner to regulate cardiovascular function. This group found a number

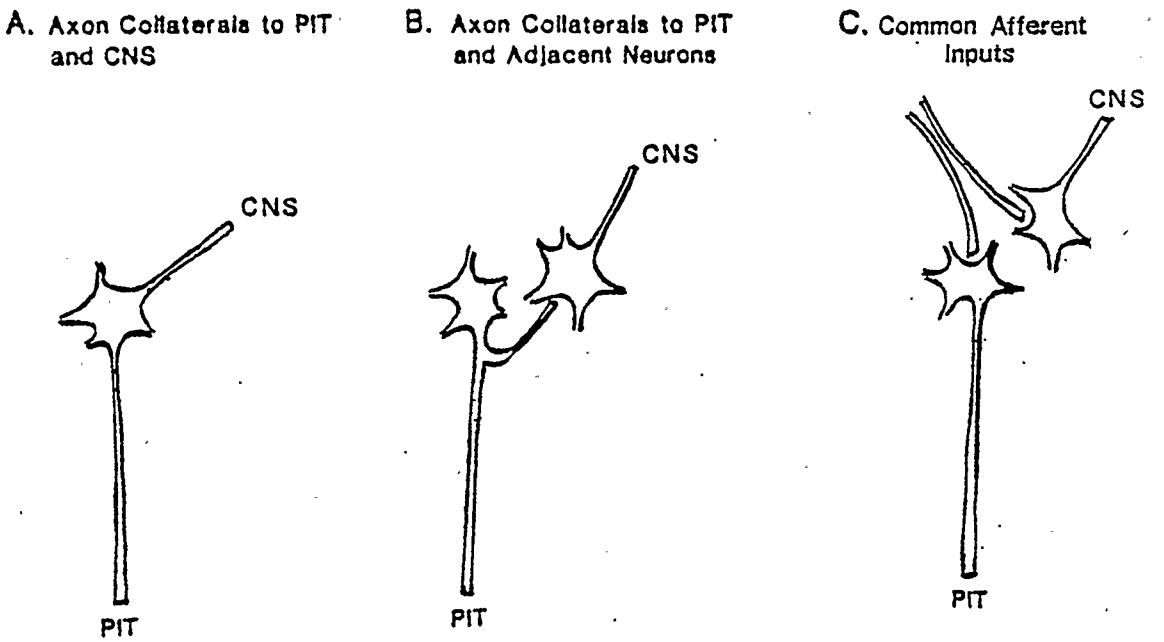


Figure 1. Diagram showing three possible mechanisms whereby neurons of the PVN could cause coordinated activity of two target areas (Pittman et al.,1982b).

of centrally projecting PVN neurons can be activated concurrently with magnocellular PVN neurons that project to the PP. The fact that these magnocellular neurons are activated in response to baro- and osmoreceptor inputs makes it likely that these same influences could also stimulate centrally projecting neurons. In fact, central and neurohypophyseal PVN projections have both been shown to respond to peripheral hypovolemic and osmotic changes (Lawrence and Pittman,1985).

Although the PVN is anatomically compartmentalized as discussed previously, neuroendocrine and autonomic responses to particular stimuli require integrated neurological activity that must be coordinated between and within these neuronal populations. Three possible mechanisms have been proposed by Pittman et al. (1982b.) whereby these two projection systems could act in a coordinated manner (See Fig.1):

a. Axon collaterals from one PVN cell to both the neurohypophysis and central sites. A small number of PVN cells have been found which project centrally and to the PP simultaneously (Yamashita et al.,1983; Zerihun and Harris,1983). Also, Sofroniew and Glasman (1981) observed using neuromorphological analysis of magnocellular PVN neurons, that some of these cells have more than one axon and some exhibit extensive axon collaterals.

b. Axon collaterals from one PVN cell project to either the neurohypophysis or to central sites, and also to adjacent PVN neurons. In this way, stimulation of one system would result in activation of the other system. Using Golgi-like

impregnations, van den Pol (1982) described axon collaterals of parvocellular neurons that ramify locally and appear to contact the dendrites of cells in both the magnocellular and parvocellular divisions. Also, Sofroniew and Glasman (1981) observed short collaterals with smaller diameters than the parent axons, and these collaterals give evidence for local interactions between cells of the two major divisions of the PVN and supports this means for integrated output of the PVN. Indeed, Janiak et al. (1989) found that centrally released AVP stimulates the release of AVP from the neurohypophysis.

c. Common afferent inputs onto different populations of peptidergic neurons in the PVN, resulting in simultaneous stimulation of both types of projections.

Given the evidence above, it is possible that PVN projections could act simultaneously within the central nervous system and the periphery to provide coordinated neural and endocrine responses to certain homeostatic perturbations involving the cardiovascular system (Riphagen and Pittman,1986).

II. HYPOTHESIS

Central projections from the paraventricular nucleus of the hypothalamus act in a coordinated manner with projections from the PVN to the posterior pituitary to aid in the regulation of the cardiovascular system.

RATIONALE FOR RESEARCH

The role of the neurohypophyseal PVN projections in regulation of the cardiovascular system is well established. However, the role of PVN projections to areas within the CNS remains obscure. If it could be shown that these neurons responded to a particular input, this could be preliminary evidence that the neuron is involved functionally. Experiments were therefore designed to answer the following question:

Do cells of the PVN which project to the VLM and NTS respond to the same stimuli as neurohypophyseal neurons of the PVN?

Most of the literature on electrophysiology of the PVN and PVN anatomy has been performed on male rats, and thus this was the animal of choice for this study. The VLM and NTS were chosen as central sites of study because they are intricately involved in cardiovascular regulation and they receive afferent input from the PVN.

III. METHODS

Experiments were performed on male Sprague-Dawley rats weighing 325-425g. Each animal was initially anaesthetized with halothane in an enclosed container. A tracheotomy tube was introduced and secured to overlying muscles. The animal was then artificially ventilated with oxygen and halothane for the duration of the experiment. Halothane was the anaesthetic chosen in this study because it provides smooth induction of anaesthesia and allows the animal to be maintained at a constant level of anaesthesia for several hours while having minimal effects on autonomic, cardiovascular and CNS function (Stimpfel and Gershey,1991).

Polyethylene catheters (PE50) were inserted into the right femoral artery for blood pressure recording and into the right femoral vein for drug administration and venous haemorrhage. Arterial pressure was measured and recorded throughout each experiment with a Gould-Statham pressure transducer and Gould polygraph recorder. Depth of anaesthesia was maintained at a level where no pinch or corneal reflexes could be evoked. Mean arterial blood pressure (diastolic pressure + 1/3 pulse pressure) at this level of anaesthesia was found to be approximately 90 mmHg which is consistent with observations in our laboratory for animals surgically anaesthetized with urethane. The ability to monitor arterial blood pressure and maintain it at this level allowed for sufficient and stable delivery of the anaesthetic for several hours. Rectal temperature was monitored and maintained between 37 and 38° C by a feedback-controlled heating pad. Random sampling of arterial blood samples for

samples for analysis of PO_2, PCO_2 and pH levels were taken and analysed using an Instrumentation Laboratory 1304 pH / Blood Gas Analyzer. These parameters were kept at approximately 140 mmHg,40 mmHg and 7.4, respectively, whenever possible by adjusting respiration.

Animals subjected to NTS stimulation were paralysed with Pancuronium Bromide (2 mg/ml given 0.1 ml intramuscularly as required) to prevent movement due to stimulation of adjacent motor reflex pathways, such as the medial and spinal vestibular pathways. Following such treatment, it was not possible to determine the depth of anaesthesia due to its paralysing effect on motor reflexes. Two methods were used to determine if the animal was surgically anaesthetized when this muscle relaxant was used:

(1) Anaesthetic level was kept at the same level (2.0 on the vaporizer) as was found adequate to maintain surgical anaesthesia in other experiments not utilizing NTS stimulation.

(2) Continuous monitoring of blood pressure was carried out and if it rose above 90 mmHg (a level maintained in anaesthetized, non paralysed animals), anaesthetic levels were increased until pressure was reduced to 90 mmHg.

The animals were placed in a stereotaxic instrument in a prone position and the skull was partially removed. Stereotaxic coordinates (Paxinos and Watson,1982) were used to aim bipolar stimulating electrodes (insulated with Q-Dope except at the very tip) at central sites known to be involved in cardiovascular regulation and receive projections from the PVN. These central sites included the ventrolateral

medulla (VLM; Dampney et al.,1985; Swanson and Sawchenko,1983) and the nucleus tractus solitarius (NTS; Yamashita et al.,1984; Rogers and Nelson,1984; Vallejo et al.,1984). A concentric bipolar stimulating electrode (Kopf instruments SNEX 100) was similarly implanted into the posterior pituitary. These electrodes were used to apply monophasic electrical pulses (15-40V, 0.2msec duration at 1-2 Hz). Single unit activity was recorded extracellularly in the PVN using a glass micropipette (5-10 MOhm impedance) filled with 2% pontamine sky blue dye marker in 0.5 M sodium acetate. Single units in the PVN were identified as projecting to the PP, VLM or NTS if they were activated antidromically by electrical stimulation of either the PP, VLM or NTS, respectively. Antidromic activation was established based on the following criteria (Pittman et al.,1986):

1. constant latency of evoked spikes
2. high frequency following of the evoked spike
3. collision of evoked and spontaneous spikes whenever possible

The activity of single identified units in the PVN was displayed on a storage oscilloscope and photographed. A variable voltage gate was utilized to select individual action potentials and the output was directed to a Digital computer (MINC-II) for spike train analysis. The output of this variable voltage gate was also directed to an integrator for output to a chart recorder to display firing frequency. A diagram of the experimental setup is given in Fig. 2. The identification of each antidromically activated cell in the PVN was then attempted by observing the cells firing response to one or more of the following stimuli:

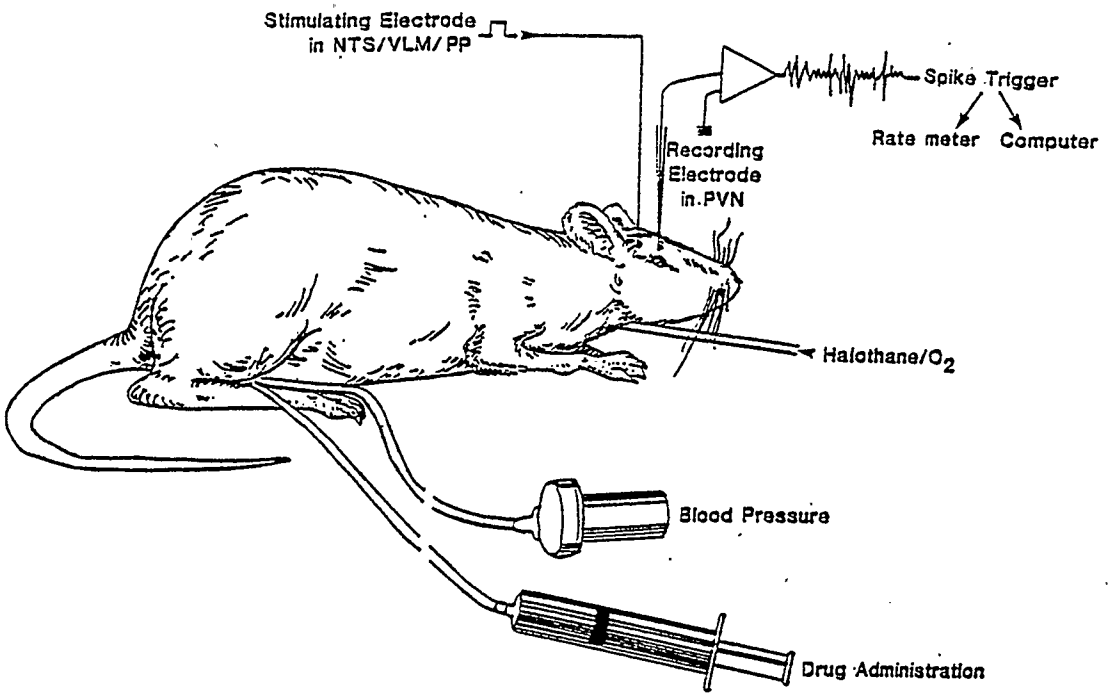


Figure 2. Diagram of the experimental model used in this study. PP = Posterior Pituitary, VLM = ventrolateral medulla, NTS = nucleus tractus solitarius, PVN = paraventricular nucleus of the hypothalamus

a.Blood pressure alterations. The PVN receives baroreceptor information andis thus involved in maintenance of blood pressure through its projections to the PP and by its central projections. Blood pressure changes are known to selectively modulate AVP release from these projections rather than OT release. An increase in blood pressure has been shown to decrease PVN-PP cell firing while the converse is true for decreased blood pressure. Consequently mean arterial blood pressure was increased to 180mmHg by iv administration of the alpha-adrenergic agonist methoxamine (0.4 mg/ml) and/or reduced to 40 mmHg by iv injection of sodium nitroprusside. Blood pressure alterations of this magnitude were previously reported to modulate the activity of PVN-PP neurons (Brown and Guyenet,1984).

b. Haemorrhage. Atrial receptors sense alterations in blood volume and transmit this information to the PVN. When blood volume is decreased, the PVN neurons are activated so that more V is released, which has been found important for blood volume recovery (Johnson et al.,1988). The decrease in blood volume also stimulates OT neurons, but the function of this is less clear. In an attempt to determine if cells were involved in this pathway, blood volume was decreased by withdrawing approximately 4 ml of venous blood (or arterial blood if the venous catheter was blocked) from the animal, over a period of 2 minutes into a heparinized syringe. The blood was held for 3 - 5 min. and then returned to the animal over a period of 1 min. Activity of the PVN cell recorded from was monitored during these physiological manipulations.

c. CCK administration. Intravenous injection of sulfated cholecystokinin

octapeptide (CCK-8) in doses of 20μg/kg, dissolved in 0.9% bovine serum albumin to prevent stickiness, were given intravenously and cell activity monitored. This manipulation has been shown to selectively activate OT cells (Renaud et al.,1987) via activation of receptors located on fibers of the gastric vagus (Zorbin et al.,1981). Thus, by injecting CCK and monitoring cell activity, putative oxytocinergic cells can be identified.

d. Osmotic changes. Cells in the PVN are activated by increases in plasma osmolality. Both OT and AVP neurons are excited by this stimulus. Thus, hypertonic saline (1.5M, 1.5ml) was injected intraperitoneally to increase plasma osmotic pressure (by approximately 30 mOsm) in order to activate PVN neurons. (Dunn et al.,1973)

e. Angiotensin II administration. Intravenous injection of angiotensin II (AG) has been found to activate both vasopressinergic and oxytocinergic neurons in the PVN and SON (Akaishi et al.,1980). Central AG receptors are found in sites such as the subfornical organ, which in turn, is thought to send excitatory projections to these hypothalamic nuclei. Intravenous injections of AG (100ng) were given to the animal in order to activate PVN neurons.

Physiological manipulation and observation of the activity of the cell allowed for identification of what the recording cell is sensitive to. The results of single PVN cells were compared to other PVN neurons to examine the uniformity of PVN responses to various stimuli.

of 7 microamps for 8 - 10 min through the recording electrode. The brain of each animal was then perfused through the heart with 0.9% saline followed by 4% neutral formalin. Subsequently, the brain was blocked, frozen and coronally sectioned at 60 μm on a sledge microtome. Each section was then be stained with neutral red and recording and stimulating electrode sites were localized by light microscopy.

ANALYSIS OF DATA

Data obtained by extracellular electrophysiological recordings of neurons in the PVN was difficult to analyze. This is because the cellular activity of the baseline without any stimulation, is variable. Thus, a true change in cellular activity due to a stimulus must be distinguished from this variation in baseline activity. Initially, the extent of baseline variation was determined for each group of PVN neurons projecting to different areas, i.e. the NTS, VLM and PP. This was calculated in the following manner.

First, mean cellular activity (spikes/sec) for each neuron was determined for the periods prior to the stimulus. The activity of the cell for the minute immediately prior to the onset of the stimulus was defined as the control minute, and activity for the minute preceding this was the precontrol minute. The activity of the precontrol minute was then calculated as a percentage of the control minute. This percentage value was designated to be the variability in baseline prior to the stimulus. For example, if the precontrol minute was exactly the same as the control minute activity, the percentage value of precontrol/control would be 100%, showing that there is 0

example, if the precontrol minute was exactly the same as the control minute activity, the percentage value of precontrol/control would be 100%, showing that there is 0 variation in this baseline for this period. However, if the precontrol minute was 8 Hz and the control minute was 10 Hz, the percentage would be 80% and there would be 20% variation in this baseline reading.

This percentage value was calculated for each prestimulus period of the corresponding neuronal group, projecting to either the PP (n = 88), NTS (n = 38) or VLM (n = 41) and a mean ± standard deviation for each group of cells was calculated.

These three groups were treated separately as normally distributed populations. For each stimulus performed on a neuron fitting into one of the 3 groups, the maximal cell activity change corresponding to a stimulus was taken as a percentage of the average baseline prior to this stimulus ((control + precontrol activity)/2). If the cellular activity resulting from application of a stimulus was more than 3 standard deviations away from the average baseline activity, a response was said to occur. This threshold value of 3 standard deviations was chosen because in a normal population, more than 99% of the data occurring by chance is found within 3 standard deviations of the mean (here, the baseline). Thus, if cellular activity is altered by more than 3 standard deviations from mean baseline activity, we can be more than 99% sure that the change is a response to the stimulus rather than due to natural variation of baseline activity. Examples of this are shown below:

PP S.D. = 6, therefore 3 S.D.'s = 18%. Thus, any of the stimuli which

altered cell activity by more than 18% of the average baseline activity was considered a response if it occurred in a corresponding period after or during the stimulus.

VLM S.D. = 5.5, therefore 3 S.D.'s = 16.5%. Any stimulus which results in a change in cellular activity by more than 16.5% is considered to constitute a response.

NTS S.D. = 5.8%, therefore 3 S.D.'s = 18%.

Analysis of this type was not feasible for phasically firing neurons due to the great variation of cellular activity shown by these cells. A review of the literature did not reveal any type of analysis useful for defining a response to a stimulus by a phasic cell, other than a specific correlation of cell activity to the stimulus, and also that the stimulus reproducibly evokes the same response. Thus, to determine if a response occurred to a stimulus in a phasically firing neuron, the criteria of an obvious and specific association of the stimulus to the altered firing must be observed. Also, if the response could repetitively be evoked by the stimulus, the change in activity was defined as a response.

IV. RESULTS

In each experiment performed, resting mean arterial pressure was approximately 90 mmHg, which is in agreement with similar experiments performed on urethane-anaesthetized rats (Brown & Guyenet, 1984). Arterial pH and PCO_2 were kept near 7.4 and 40 mmHg, respectively. PO_2 was usually much higher than the normal value of 140 mmHg (up to 220 mmHg).

Histological examination verified the placement of the recording and stimulating electrode in each experiment. A photograph demonstrating representative recording sites within the PVN is given in Fig. 3. The vicinity of the VLM and NTS stimulating electrodes is shown in Fig. 4.

Extracellular recordings were made from 56 animals which met the criteria of adequate health during the experiment and for which the loci of stimulating and recording electrodes could be verified. A total of 176 PVN neurons were found to conform to the 3 accepted criteria for antidromic invasion (Fig. 5). That is, they showed constant latency to onset of the evoked potential after stimulation, high frequency following at 200 Hz, and cancellation of the evoked potentials with spontaneously occurring orthodromic potentials in spontaneously active neurons. Stimulation of the PP area elicits action potentials which travel from the axon terminal back up to invade the soma, the antidromic potential. This activity is recorded in the PVN if that is the site of origin of this cell's axon which is excited. A stimulation and recording paradigm such as this would have a constant time

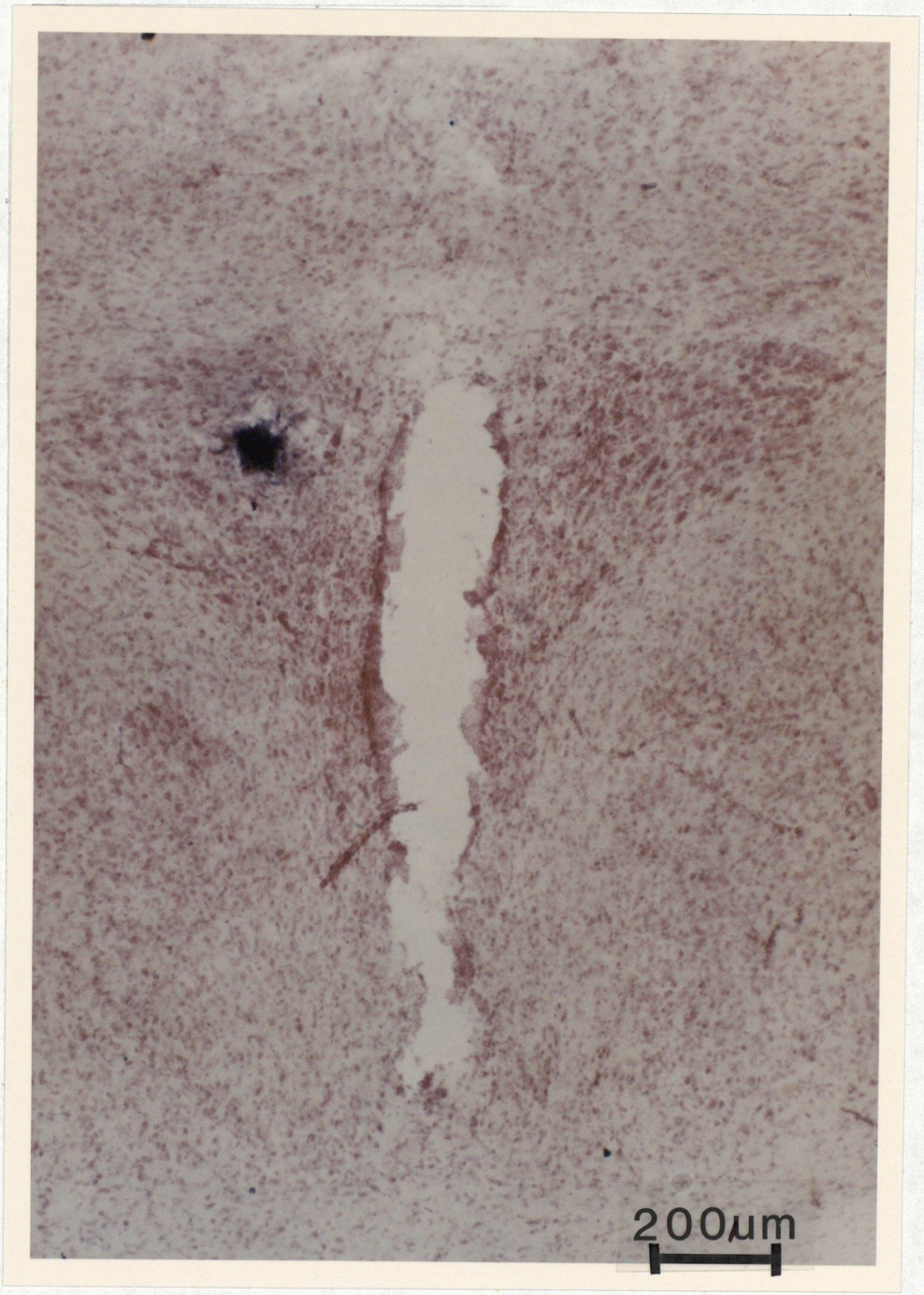


Figure 3. Photomicrograph of a coronal section of the paraventricular nucleus of the hypothalamus, demonstrating a representative area for recording. The large blue stained area on the left is a dyespot from the recording electrode.

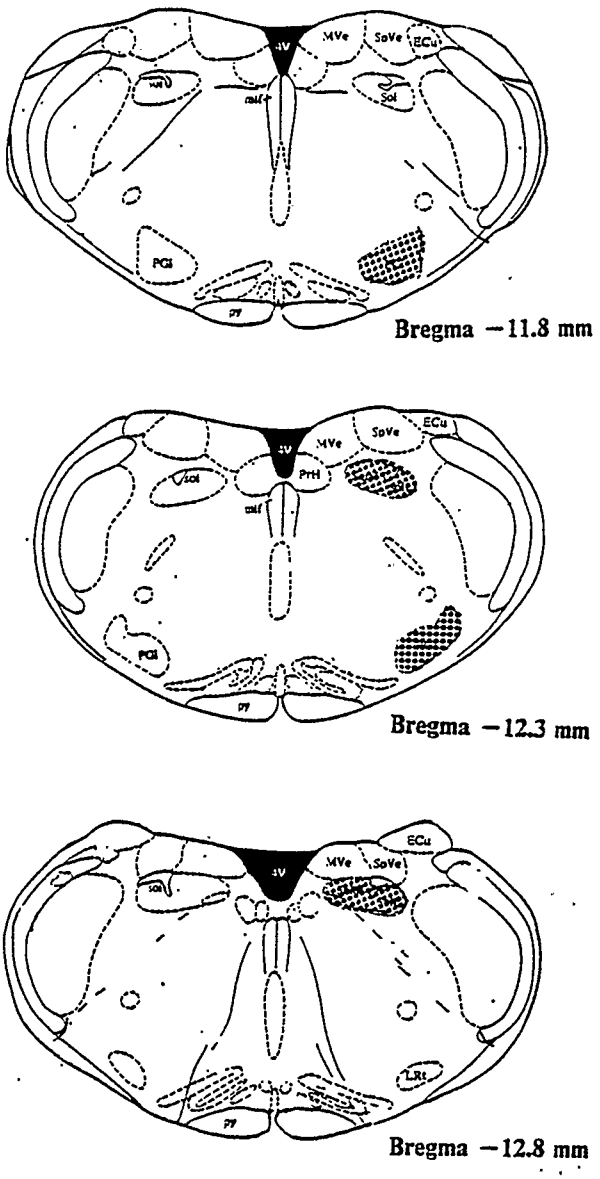


Figure 4. Location of stimulation sites in the ventrolateral medulla (nucleus paragigantocellularis lateralis, PGi) and in the NTS (sol) in schematic sections adapted from Paxinos and Watson (**1982**). Stippled areas represent areas where the stimulating electrode tracts were found.

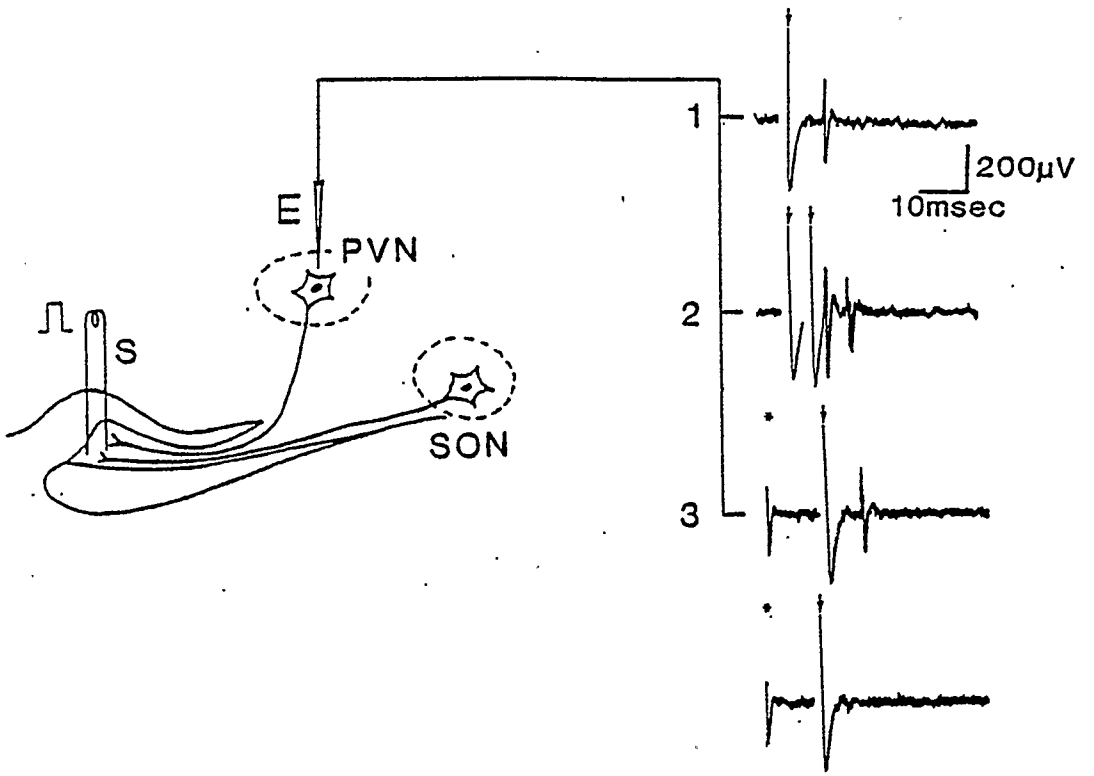


Figure 5. Diagram showing microcircuitry and the three criteria required to antidromically identify a cell in the PVN. This drawing demonstrates an experimental design whereby a stimulating electrode (S) is placed in the posterior pituitary (PP), and a recording electrode (E) is in the paraventricular nucleus of the hypothalamus (PVN). PP stimulation excites an axon which has its cell body near the PVN recording electrode, and the action potential travels antidromically to invade its soma and is recorded. The three antidromic criteria are shown on the right. Number 1 demonstrates constant latency of the evoked antidromic potential. Number 2 demonstrates the requirement of high frequency following for the antidromical potential. Finally, number 3 shows that if the cell is spontaneously active (asterisk), the antidromic potential must collide with the spontaneous activity if it is elicited within the antidromic latency.

between stimulation and recording because the electrical activity travels through a neuron at a constant velocity, and no synapses are crossed (1). Also, because the action potential does not have to cross a synapse, stimulation at high frequencies (100-200 Hz) is followed consistently by antidromic potentials (2). Finally, if the neuron being recorded from is spontaneously active, the antidromic potential will collide with this spontaneous action potential which is travelling in the opposite direction (3). This would occur if the antidromic potential was elicited within the time it takes for an action potential to travel from the soma to axon terminal, or vice versa (the antidromic latency).

Of the 176 PVN neurons identified, 58 PVN neurons were antidromically activated following stimulation of the PP only, 46 were antidromically invaded following stimulation of the NTS only, 68 following VLM stimulation only. Additionally, 3 neurons were antidromically invaded following stimulation of both the PP and brain stem nuclei.

A. Neurohypophyseal PVN Projections

Of the 58 neurons antidromically invaded only by the PP (PVN-PP cells), 9 showed phasic activity, 18 were silent, and 31 were continuously active. The voltages required to activate these neurons varied from 1-28 V, the mean voltage for activation being 10 V. The antidromic latencies for neurohypophyseal cells varied from 5 - 25 msec with a mean of 12 msec. For a straight line distance between

stimulating and recording electrodes of approximately 6mm, this gives conduction velocities of less than 1m/sec, suggesting that the axons are unmyelinated.

Among neurons in the region of the PVN which were antidromically invaded by PP stimulation, 46 were further examined for responses to cardiovascular and other stimuli. Of these cells, 21 were sensitive to blood pressure alterations, 6 of 16 tested responded to haemorrhage, 5 of 9 tested responded to hypertonic saline injection, and 13 of 24 tested responded to iv CCK administration. Four of the cells that responded to CCK administration did not respond to alterations in blood pressure, and thus were considered OT cells (Renaud et al.,1987). In addition, 2 of 3 cells tested were found to respond to angiotensin II (AG) administration.

Methoxamine injection increased blood pressure to approximately 180mmHg. The response of most PVN-PP cells to this increase in blood pressure was a decrease in cell firing (17/18). This included stopping or decreasing the bursting interval of phasic PVN-PP neurons during elevated blood pressure, a well documented result for putative AVP neurons. For example, figures 6 and 7 are phasically firing PVN-PP cells. The cell in Fig. 6 stops its phasic burst when mean arterial blood pressure is increased to approximately 140mmHg, and resumes firing once blood pressure returns to 140mmHg and below. This response was typical of most phasically firing neurons found in this study. Alternatively, the phasic cell activity demonstrated in Fig. 7 did not stop when blood pressure was increased to levels above 180 mmHg. Instead, the bursting periods during elevated blood pressure were shortened, and interburst intervals elongated during the period of elevated blood pressure.

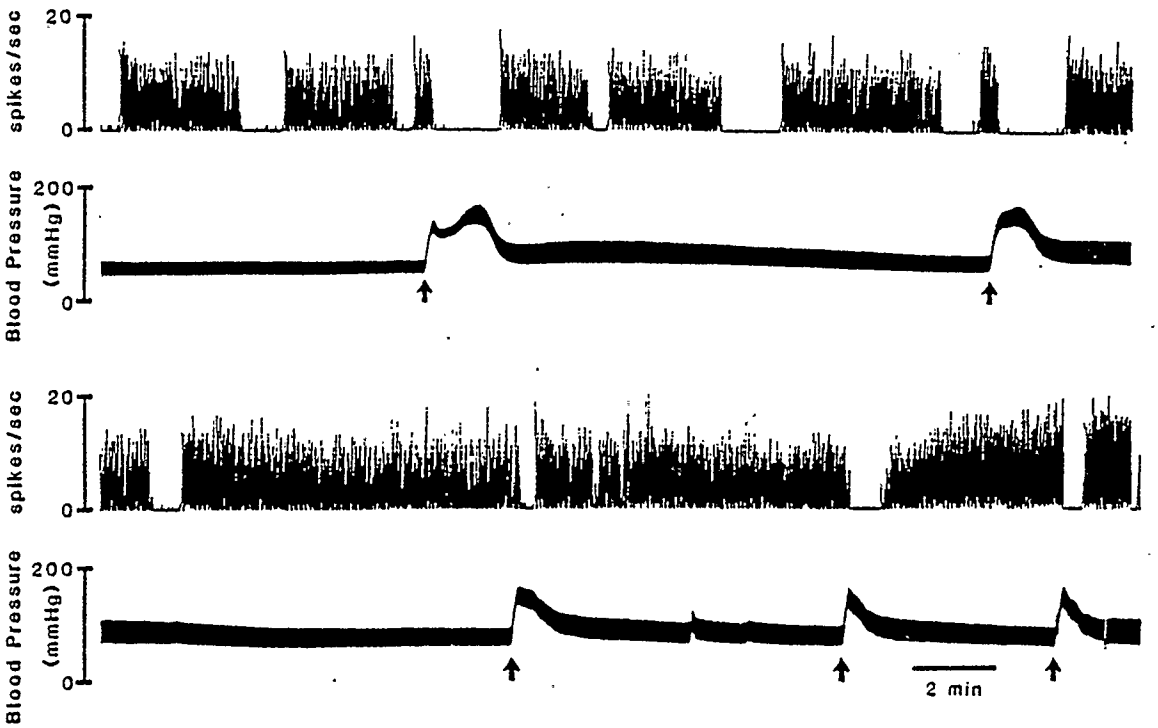


Figure 6. Rate meter records (top) and blood pressure traces (bottom) obtained from a phasic PVN-PP neuron during injections of methoxamine (arrows). Injection of methoxamine, indicated by the arrows, results in increased blood pressure and resultant arrest of a phasic burst which begins again only when blood pressure decreased to the basal level.

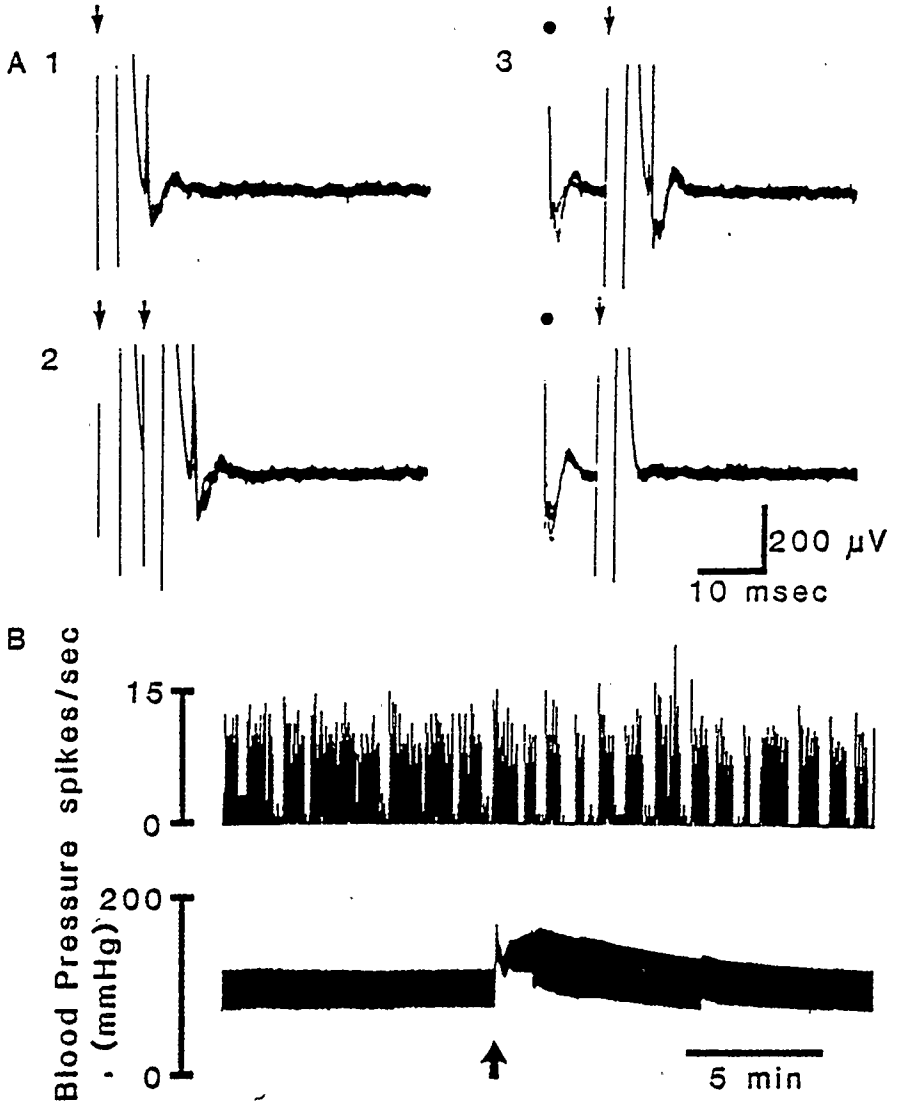


Figure 7. Antidromic criteria and firing rates of a phasic PVN-PP cell. Part A demonstrates antidromic criteria of this cell: 1. constant latency of evoked spike, 2. high frequency following of the evoked spike, and 3. collision of the antidromic potential with a spontaneous action potential (below dot). Arrows mark stimulation artifacts. Part B gives rate meter records (top) and blood pressure (bottom) traces for this cell. An increase in blood pressure by intravenous injection of methoxamine (arrow) lengthens the interburst intervals and shortens bursting periods during the duration of increased blood pressure.

Putative vasopressinergic neurohypophyseal cells of the PVN have been shown to display a multitude of different firing patterns, including phasic activity, continuous activity, or lack of spontaneous activity. One example of a continuously firing PVN-PP neuron is shown in Fig. 8. The cellular activity of this neuron was inhibited by increases in blood pressure, and this response was typical of PVN-PP neurons in this study. Note, however, that this cell did not respond to a decreased blood pressure evoked by intravenous nitroprusside (NP). Both haemorrhage and injection of nitroprusside decreased mean arterial blood pressure to approximately 40 mmHg in this study. However, some cells respond to volume changes preferentially over decreased blood pressure. Thus, it is difficult to distinguish if a cell response to haemorrhage is due to decreased blood volume or pressure. For example, the PVN neurohypophyseal cell in Fig. 9 increased its firing by more than 200% during arterial haemorrhage. Pre haemorrhage levels of firing resumed once blood was returned. This response is typical of PVN-PP neurons responding to either decreased pressure or volume. Another example of this typical response to haemorrhage is demonstrated in Fig.10. This cell is a phasic PVN-PP neuron which increased its activity during haemorrhage. This figure also demonstrates the typical excitatory response to AG and hypertonic saline injections. However, only 3 neurons were tested for AG administration and thus data was insufficient to establish the action of this substance on PVN neurons.

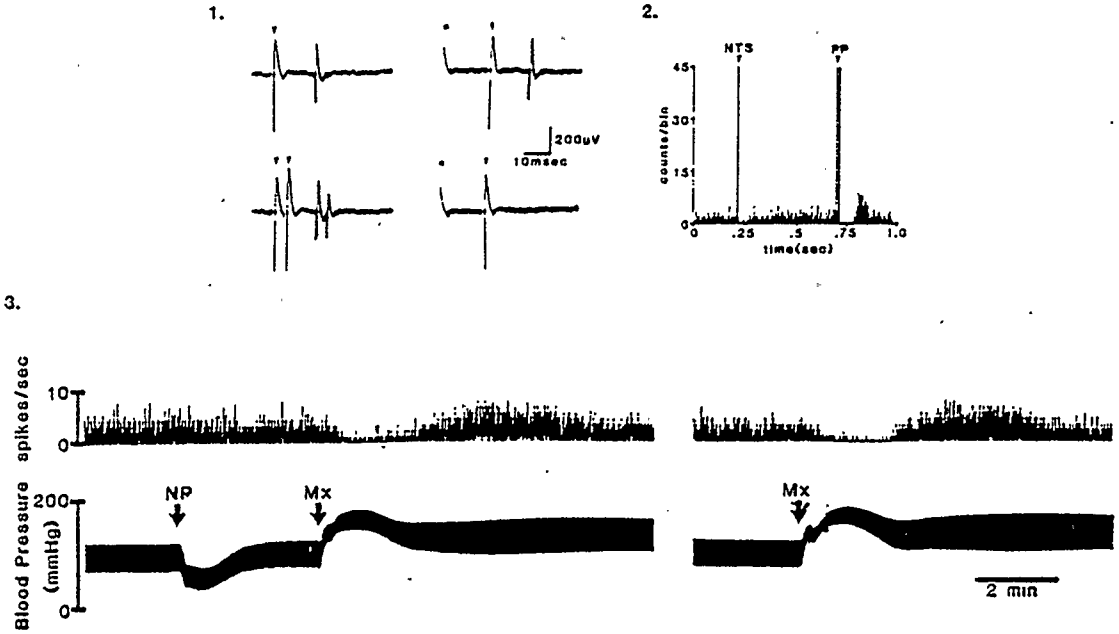


Figure 8. Antidromic criteria (1) for a PVN-PP neuron. Arrows indicate stimulus artifacts and asterisks mark spontaneous action potentials. Part 2 demonstrates a peristimulus time histogram (PISH) of this cell. A PISH is a cumulative recording of cell activity for repeated one second intervals, during which the stimulation of areas in which stimulating electrodes are placed occurs. This record gives information of the cells activity before, during, and after stimulation of these areas. The PISH shown here demonstrates that electrical stimulation of the NTS has no effect on the PVN cell being recorded from, however the PP stimulation is consistently followed by an action potential at a constant latency, the antidromic potential. Thus, a PISH helps to identify an antidromically activated neuron and determine possible orthodromic influences.

Part 3 demonstrates the cellular activity of this PVN neurohypophyseal neuron along with blood pressure recordings. Increased blood pressure due to intravenous methoxamine (Mx) inhibited this cells activity by 85% during the period of elevated pressure. However, a decrease in blood pressure by intravenous injection of sodium nitroprusside (NP) did not affect the firing of this cell.

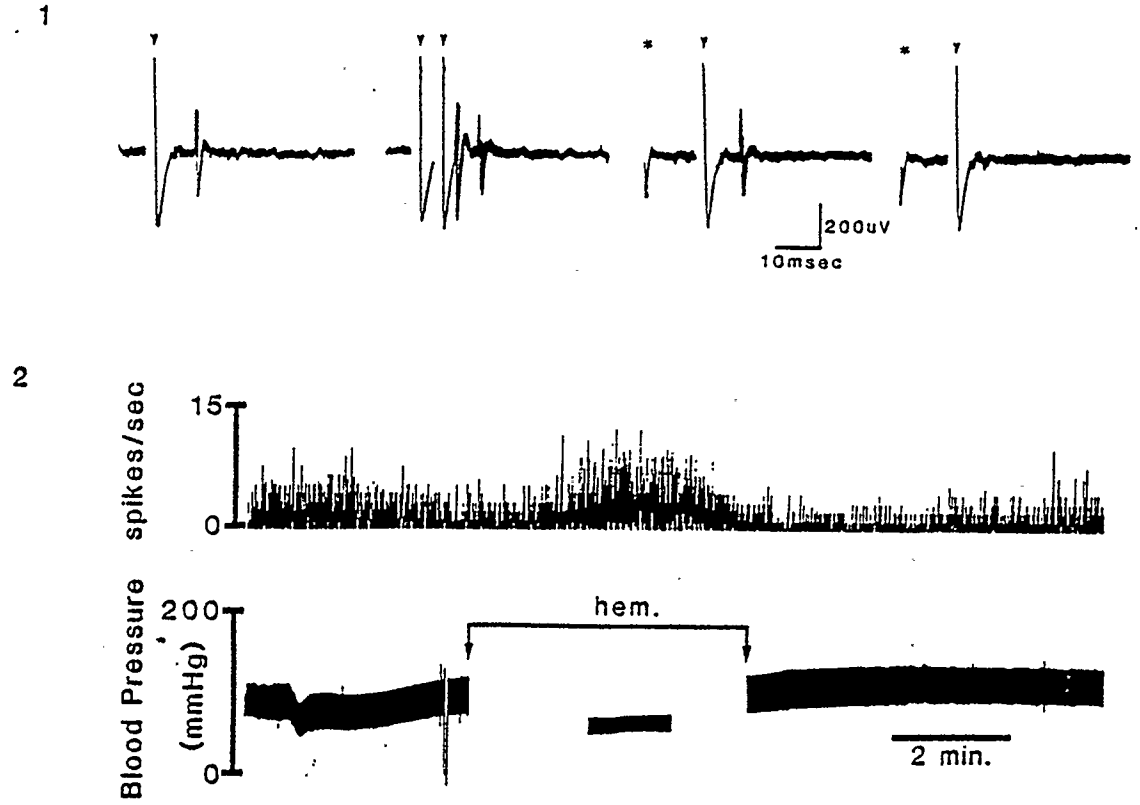


Figure 9. Antidromic criteria (1) for a PVN-PP cell. Arrows indicate stimulus artifacts and asterisks mark spontaneous action potentials. Rate meter recordings and blood pressure traces of this cell are displayed in section 2. During the period of arterial hemorrhage, this cell's activity increased by 230%.

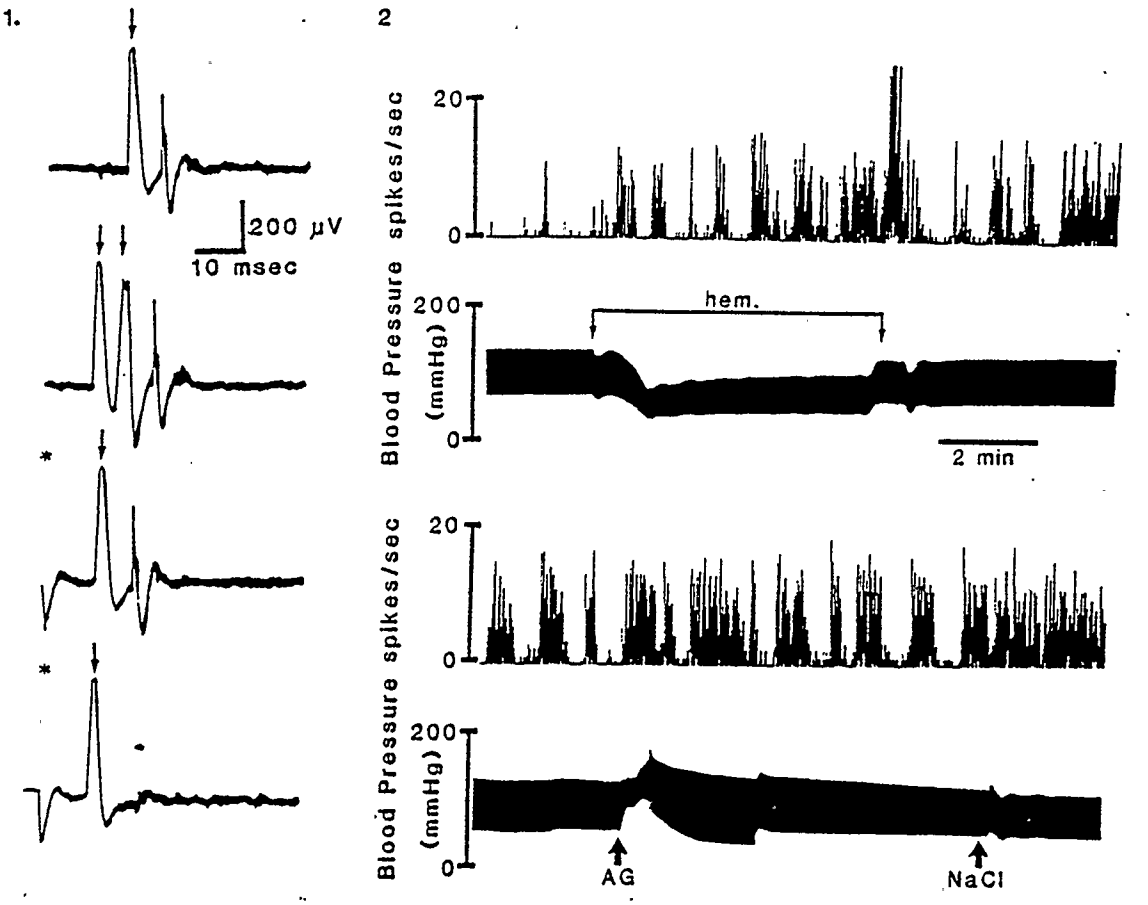


Fig. 10. Antidromic criteria for a phasic PVN-PP neuron (1), where arrows indicate stimulus artifacts and asterisks mark spontaneous action potentials. Part 2. shows rate meter recordings (top) and blood pressure traces (bottom) for this cell. A 4.0ml venous hemorrhage was performed in the period marked hem. Prior to hemorrhage, this cells activity was almost negligible, demonstrating only one phasic burst of 10 spikes per second for approximately 15 seconds. However, hemorrhaging the animal, injecting angiotesin II (AG) and injecting hypertonic saline (NaCl) activated this cell into long bursting periods with short intervals devoid of action potentials.

The characteristic response of PVN-PP neurons to peripheral CCK administration was to increase neuronal activity (9/13). This typical response is demonstrated in Fig. 11, where the activity of the cell increased by more than 600% following the initial injection. The cell identified in Fig. 12 was also activated by systemic CCK injection. This PVN-PP neuron received excitatory input from the NTS. Fig. 13 shows the responses of this cell to several stimuli, including CCK. This was excited not only by peripheral CCK, but it was also sensitive to alterations in blood pressure and increased plasma osmolality.

Fig. 14 illustrates a classical oxytocinergic neurohypophyseal PVN neuron, which was blood pressure insensitive but which was excited by peripheral CCK administration. However, a few neurons were observed in this study to be inhibited by peripheral CCK administration, as shown in Fig.15. Another cell, shown in Fig. 16, is excited by hypertonic saline injection, but is inhibited by more than 60% by CCK.

B. PVN-VLM Neurons

Sixty eight PVN neurons exhibited antidromic activation following VLM stimulation (PVN-VLM cells). Of the cells in whose activity patterns were recorded, 22 were silent, 30 exhibited continuous spontaneous activity, and none were phasically active. The voltage required to activate the neurons varied from 10-40V, with a mean voltage for activation being 22V. The latency to onset of activation

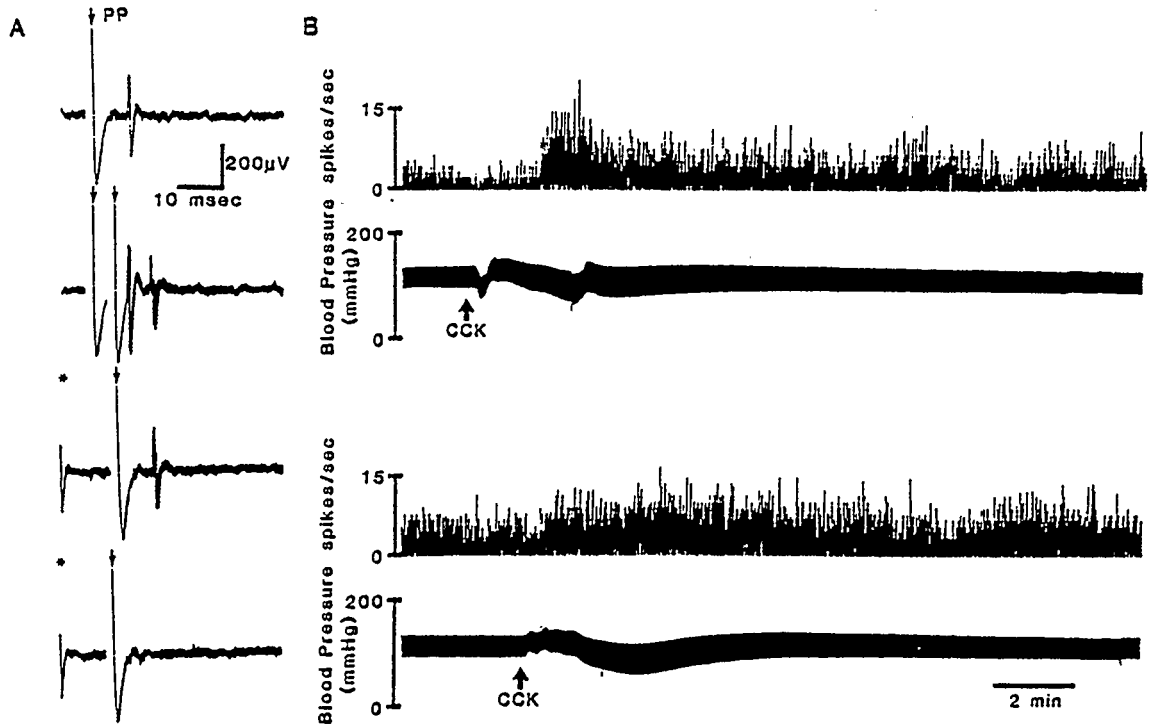


Figure 11. Diagram showing antidromic criteria (A) and rate meter records (top) and blood pressure (bottom) traces obtained from a neurohypophyseal neuron during iv injections of CCK. Injection of 8μ g of sulfated cholecystokinin octapeptide (CCK) in 0.9% BSA caused cell firing to increase by more than 600% in the first trial and 185% in the second trial. These increases occurred within 2 minutes of injection.

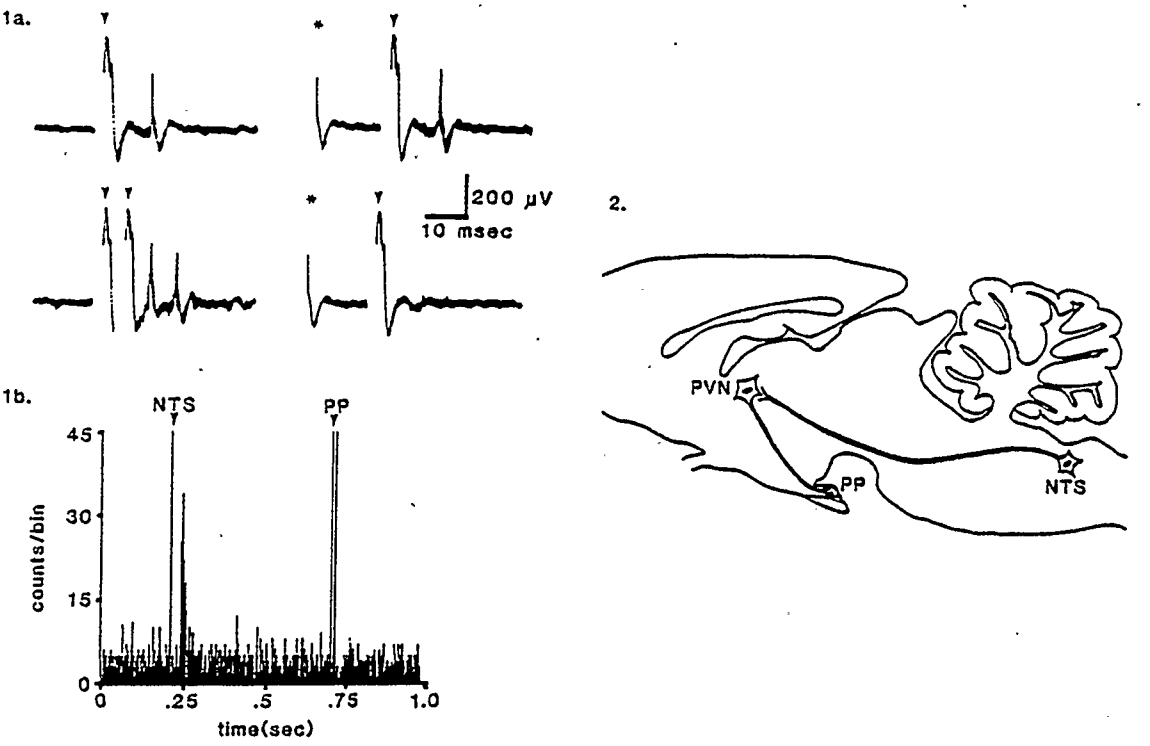


Figure 12. Antidromic criteria (1a.), PISH (1b.) and schematic diagram (2) of a PVN-PP neuron orthodromically activated by NTS stimulation.

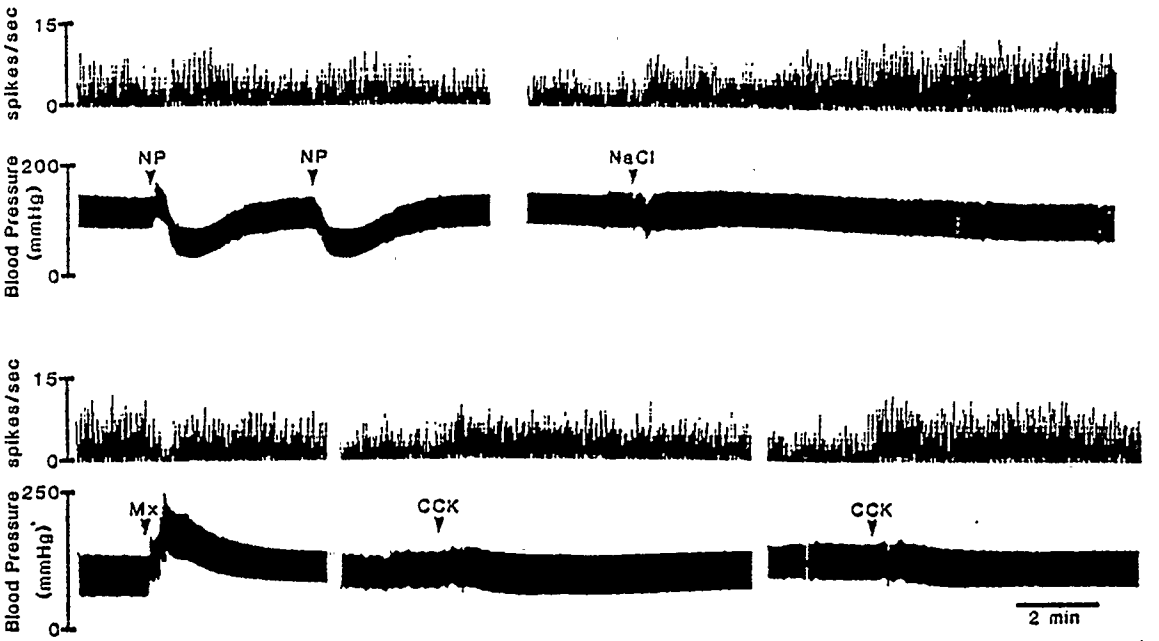


Figure 13. Rate meter recordings (top) and blood pressure traces (bottom) of the PVN-PP cell identified in Fig 12. This cell was activated by decreases in blood pressure (NP), injection of hypertonic saline (NaCl) as well as peripheral CCK administration. Methoxamine (Mx) was found to transiently decrease cell activity when blood pressures rose to 225mmHg and over.

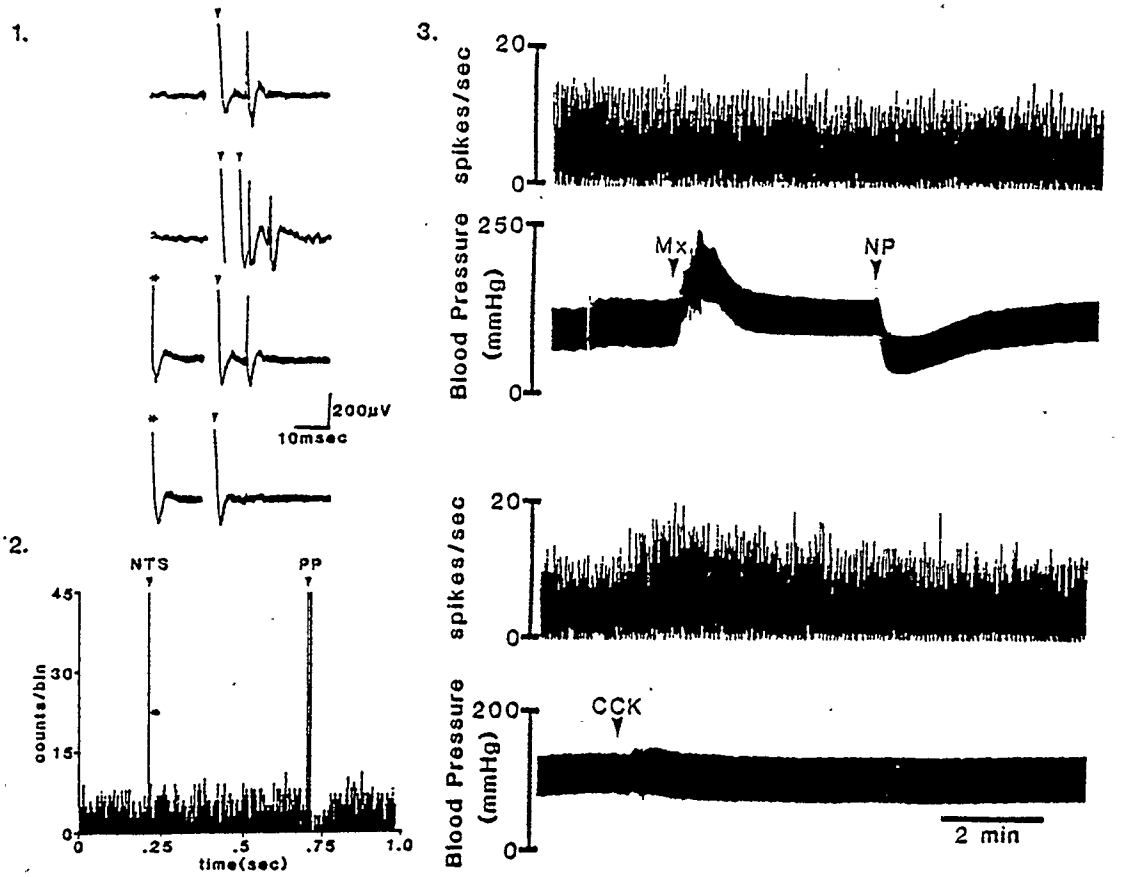


Figure 14. Antidromic criteria (1), PISH (2) and rate meter recordings with blood pressure traces (3) for a PVN-PP cell. NTS stimulation had no effect on this cells activity as shown by the PISH. Increases and decreases in blood pressure (using methoxamine (Mx) and nitroprusside (NP) respectively) had no effect on the cell firing. However, injection of cholecystokinin (CCK) resulted in an immediate increase of cell firing by 144%.

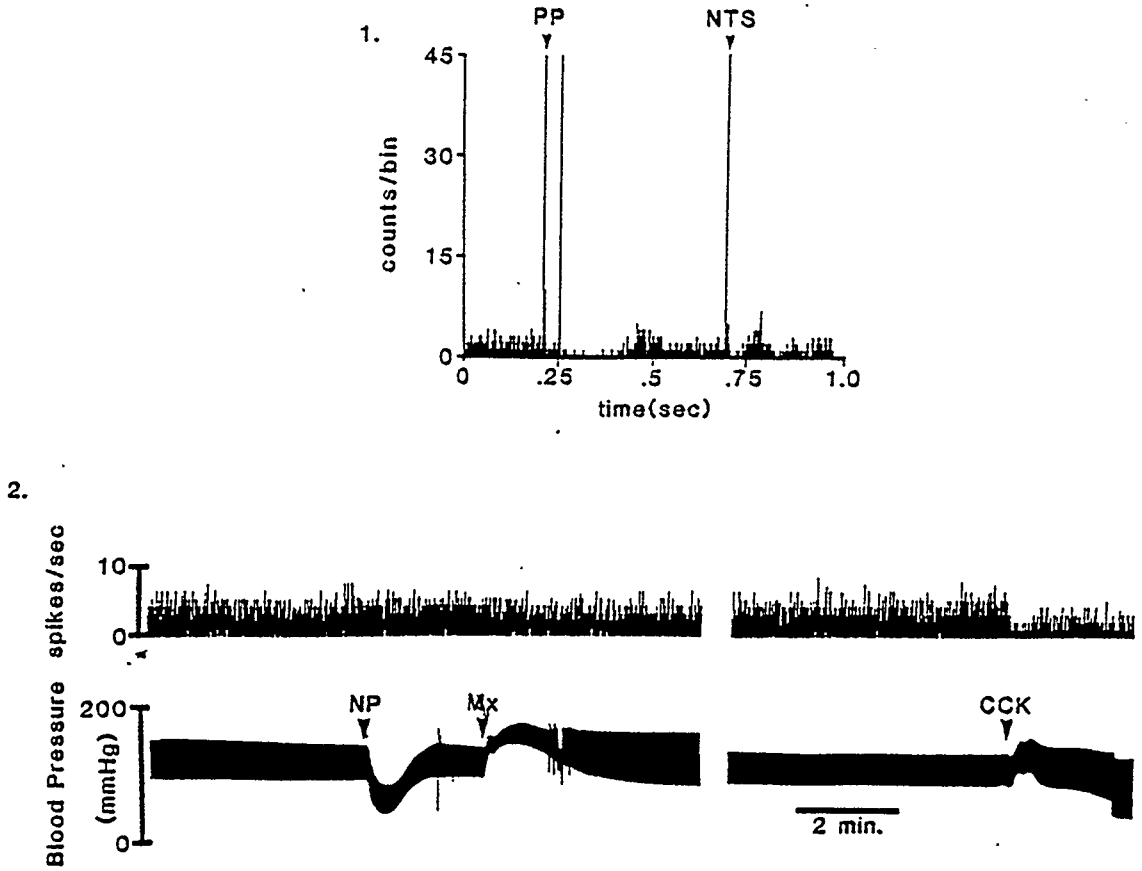


Figure 15. PISH (1), rate meter recordings and blood pressure traces (2) for a PVN-PP cell. Injections of nitroprusside (NP) or methoxamine (Mx) do not influence the cell activity, although a cholecystokinin (CCK) injection immediately reduced cell activity by 65%. The cell did not recover from the CCK injection, but rather, was unable to be recorded from approximately 3 minutes after CCK was injected.

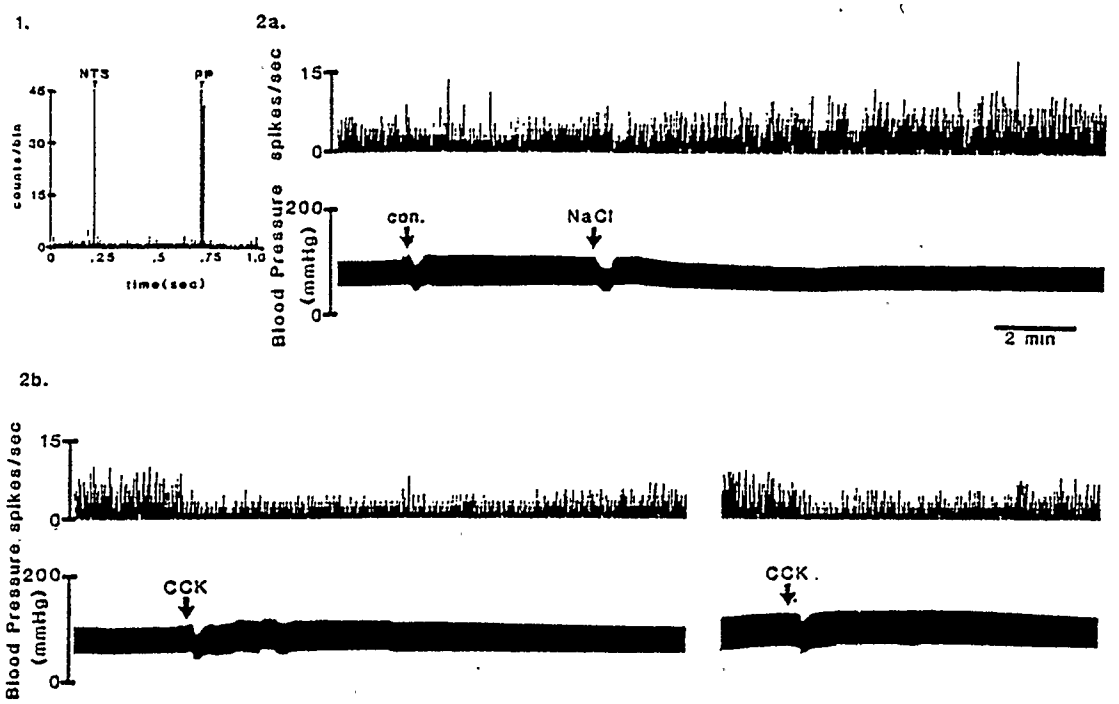


Figure 16. Diagram of a PISH (1), rate meter and blood pressure traces (2) for a PVN neurohypophyseal cell. In 2a., a 1.0 ml control injection of 0.9% saline did not alter cellular activity. However hypertonic saline injection (NaCl) increased firing activity by 168% within 10 minutes after injection. In contrast, injections of cholecystokinin (CCK, 8μg) inhibited firing to 35% of baseline almost immediately after injection.

varied from 5 to 46 msec, with an average of 22 msec. For a straight line distance between the PVN and VLM being approximately 10 mm, this corresponds to a conduction velocity of less than 1 m/sec, indicating that these axons are also unmyelinated.

A total of 41 PVN-VLM cells were observed to respond to blood pressure and other physiological stimuli. Eight of these cells were found to respond to alterations in blood pressure, all by decreasing cellular activity. Four of these neurons were inhibited by nitroprusside (NP) induced blood pressure decreases (Fig. 17). On the other hand, 4 neurons exhibited decreased cellular activity when blood pressure was increased by methoxamine (Mx) injection (Fig.18).

The information in Fig. 18 compares Mx induced hypertensive responses of PVN-PP neuron to that of PVN-VLM neurons. The PVN-PP neuron displayed typical abrupt cessation of activity in response to the blood pressure increase. In contrast, PVN-VLM neurons usually display no response to blood pressure alterations (33/41) (Fig.18b). Fig. 18c, however, displays an atypical inhibition of a PP-VLM neuron following a transient increase in blood pressure.

No PVN-VLM neurons tested showed a response to peripheral CCK administration (0/9). An insufficient number of neurons were tested for haemorrhage (1) and hypertonic saline injection (3) to be able to draw any conclusions about these manipulations.

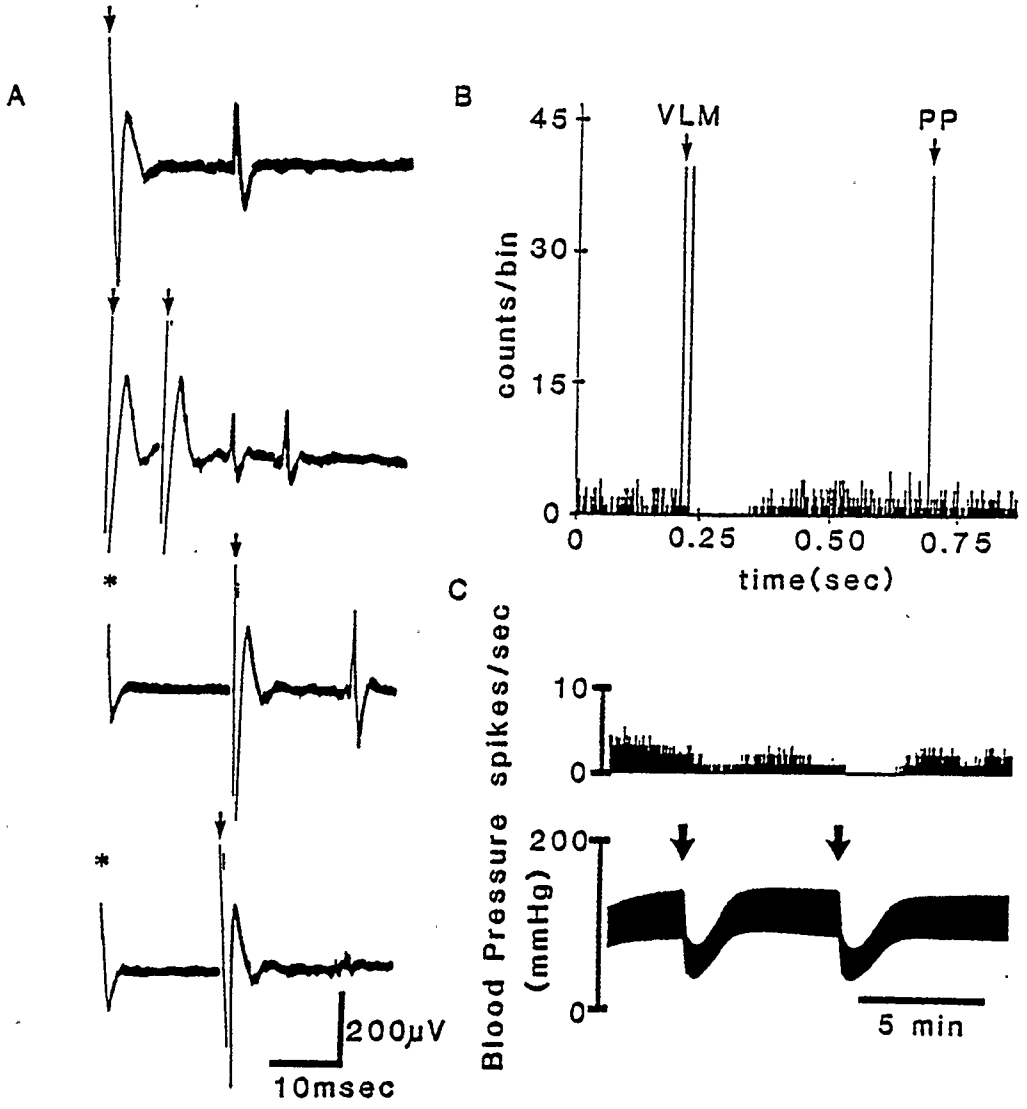


Figure 17. Diagram showing the antidromic criteria (A), PISH (B) and rate meter recordings with blood pressure traces for a PVN-VLM cell. Arrows in A indicate VLM stimulus artifacts and asterisks indicate spontaneous action potentials. In B, VLM stimulation is consistently followed by an action potential recorded from the PVN, and is followed by inhibition for approximately 1/8 of a second. Stimulation of the PP has no effect on this cell. In C, it is shown that decreased blood pressure by intravenous injection of NP (arrows) results in decreased cell firing which resumes once blood pressure recovers to preinjection levels.

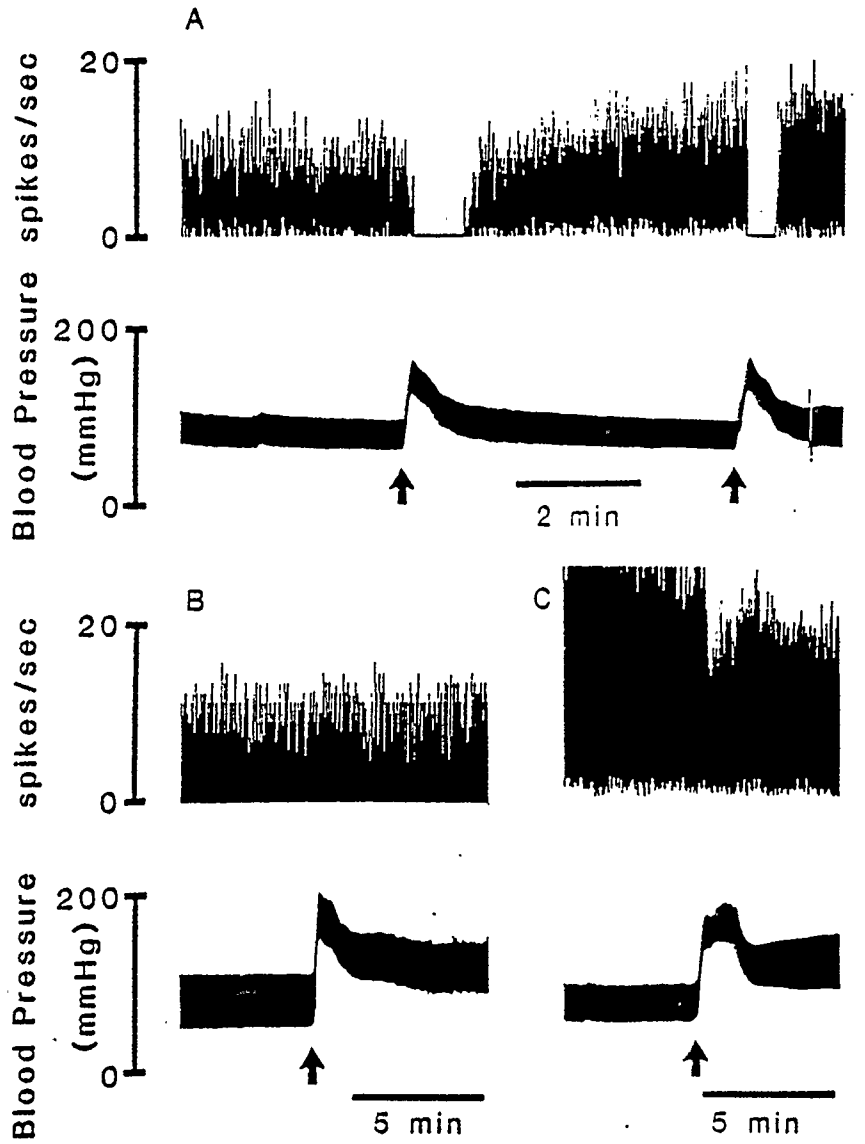


Figure 18. Rate meter recordings and blood pressure traces of three different cells which respond differently to increases in blood pressure by methoxamine (arrows). The tracings in A are from a phasic PVN-PP whose activity is arrested in mid-burst when blood pressure increased. Sections B and C are records of different PVN-VLM cells. The cell shown in B is not affected by increased blood pressure whereas the cell in C decreased its activity in response to this stimulus.

C. PVN-NTS Neurons

Forty five neurons in the PVN were antidromically activated by NTS stimulation only (PVN-NTS cells). Of the 43 neurons whose cellular activity was recorded, twelve of these cells exhibited continuous spontaneous activity, 31 were silent, and none showed phasic activity. The voltages required to activate these neurons varied from 5-46V, with a mean threshold voltage of 21V. The antidromic latency of these cells varied between 13 and 66 msec after the NTS was stimulated, with an average latency of 35 msec. This would correspond to a conduction velocity of less than 1 m/sec, indicating these neurons are unmyelinated.

Twenty-five of these neurons were tested for blood pressure alterations or other physiological manipulations. Only 2 of 23 cells tested for responses to blood pressure changes showed a response. As illustrated in Fig. 19, these PVN-NTS neurons were inhibited by decreased blood pressure due to NP injection and were unresponsive to increased blood pressure. The neuron in Fig. 19 was also inhibited by haemorrhage. This was an atypical PVN-NTS neuron because it was the only cell of 13 tested for haemorrhage that showed a response. However, both haemorrhage and NP injection decreased mean arterial blood pressure to approximately 40 mmHg in this study. Therefore, this cell possibly responded to the decreased pressure caused by the haemorrhage rather than to the decreased blood volume.

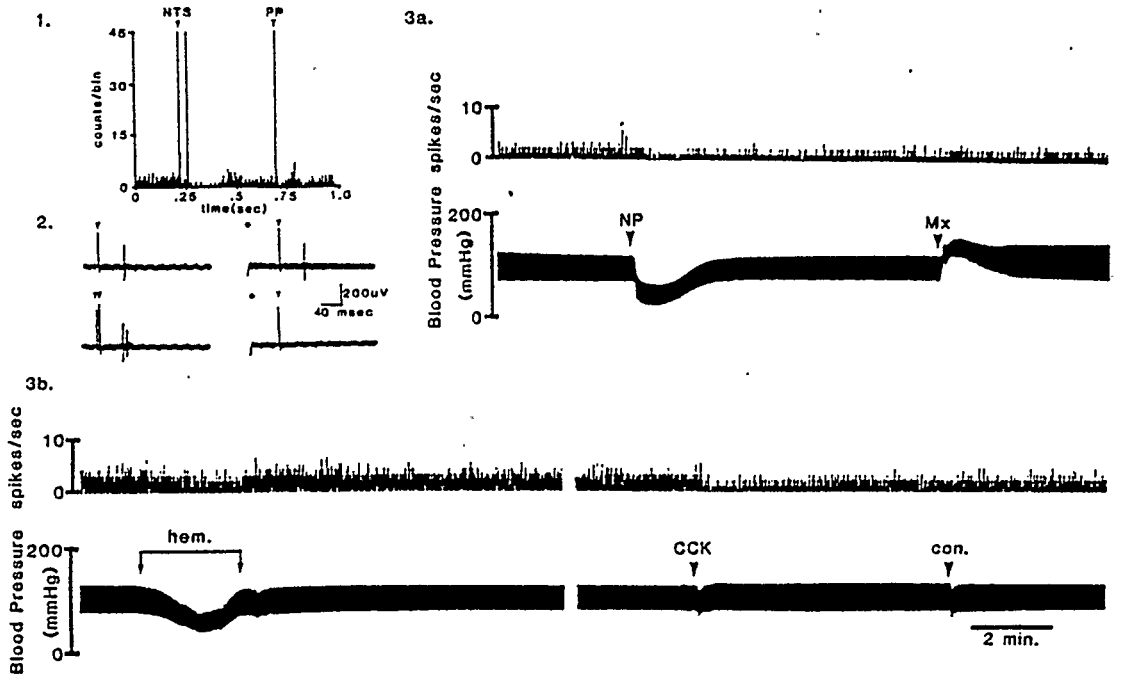


Figure 19. Diagram showing a PISH (1), antidromic criteria (2) and rate meter recordings with blood pressure traces (3) of a PVN-NTS neuron. As shown in the PISH, NTS stimulation inhibited cellular activity whereas PP stimulation had no effect (1). In C, a blood pressure decrease due to NP injection decreased cell firing by 35%, whereas increased blood pressure from Mx injection has no effect. A 4.0ml venous hemorrhage caused cell firing to decrease to 60% of baseline as blood pressure was increasing back to control levels. Injection of cholecystokinin (CCK) inhibited cell activity to 44% of control levels. A control injection of normal saline (con.) did not affect cell activity.

CCK administration was not a potent stimulus for PVN-NTS cells. Only 2 of 20 neurons tested for a response to this stimulus responded, and both decreased their activity. One of these cells is that in Fig. 19. The other PVN-NTS neuron whose activity was inhibited by CCK injection is illustrated in Fig. 20. This neuron was insensitive to changes in blood pressure, but it exhibited increased activity in response to peripheral CCK injections. This cell has responses similar to the PVN-PP neuron illustrated in Fig. 15.

D. Dual PVN Projections to the PP and Brain Stem

Although small in number, the three neurons that were found to project to both the pituitary and the medulla are extremely interesting. Each neuron was found in a different animal and was identified because it could be antidromically invaded from both sites. Stimulation from each site met all 3 criteria for antidromic activation but, in addition, the antidromic potential evoked by stimulation of one site was cancelled by stimulation of the other site. This was possible when the stimuli were applied at an interval that was less than the sum of the latencies to onset of the 2 evoked potentials.

Figs. 21-23 illustrate the data obtained from a PVN neuron which projected to the NTS and PP simultaneously. The pituitary-evoked potential had a latency of 14 msec, and the NTS evoked potential had a latency of 42 msec. The threshold for activation of this cell was 8V and 10V by the PP and NTS, respectively. Provided

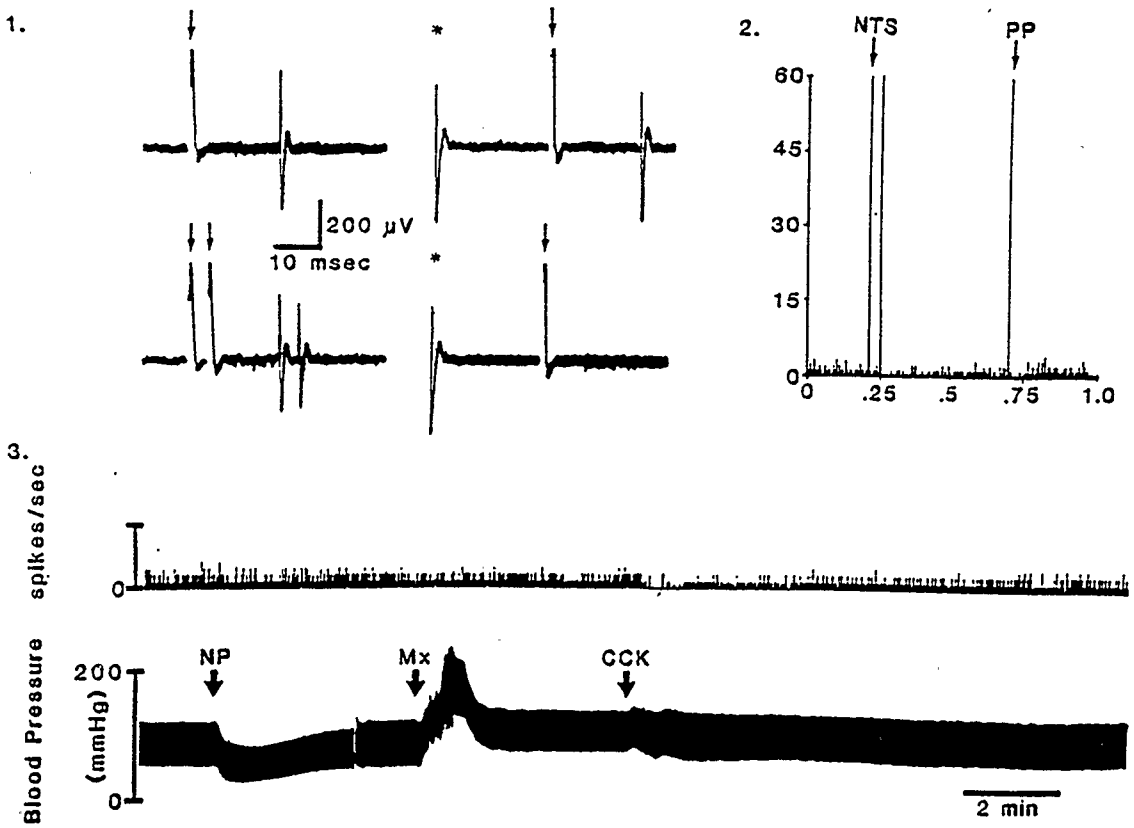


Figure 20.. Diagram demonstrating antidromic criteria (1), PISH (2) and rate meter recording with blood pressure tracing (3) for a PVN-NTS cell. This neuron was insensitive to increases and decreases in blood pressure (by injection of methoxamine (Mx) and nitroprusside (Np) respectively), although injection of cholecystokinin (CCK) decreased cell firing by approximately 80% immediately after the injection.

1.

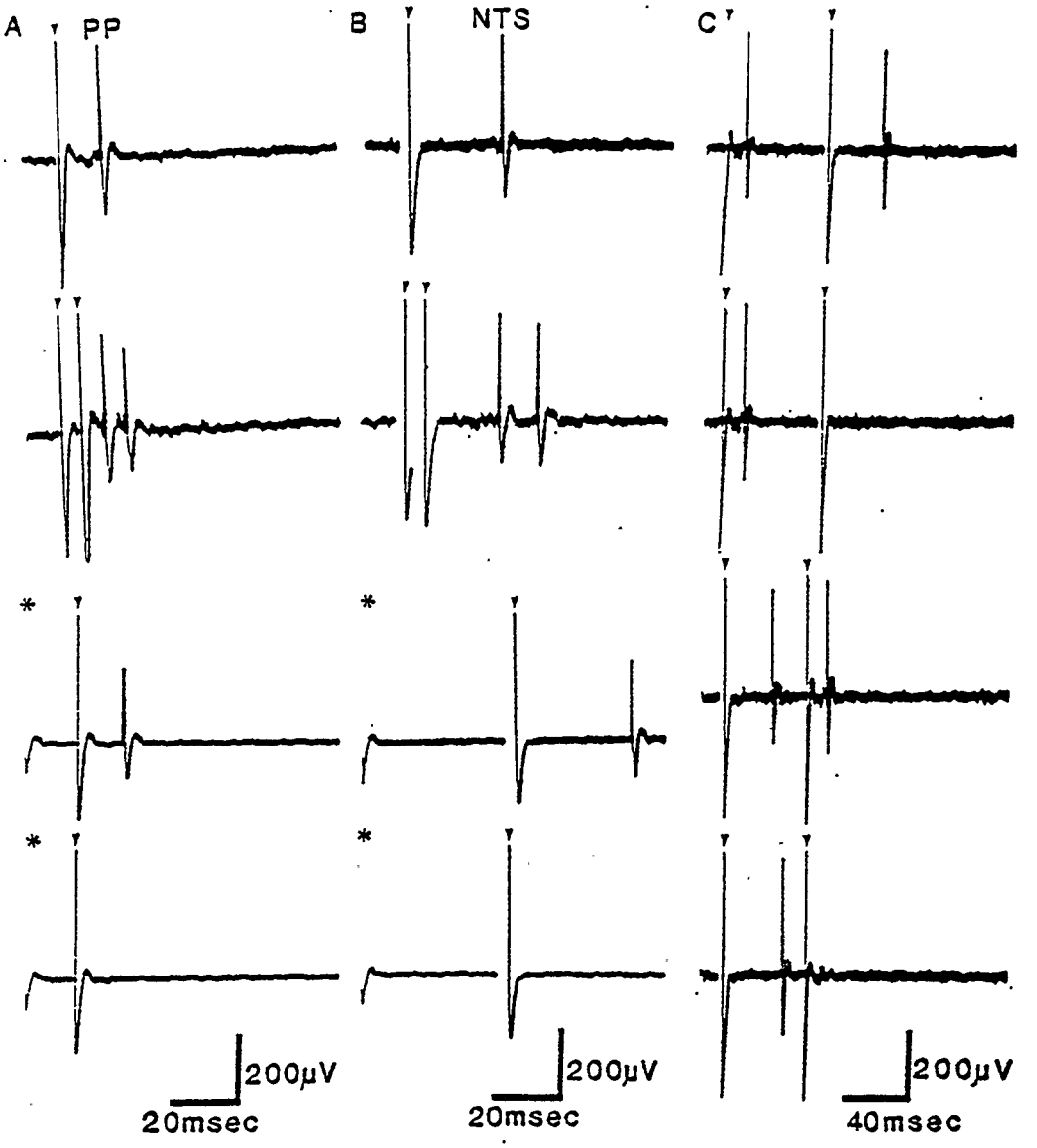
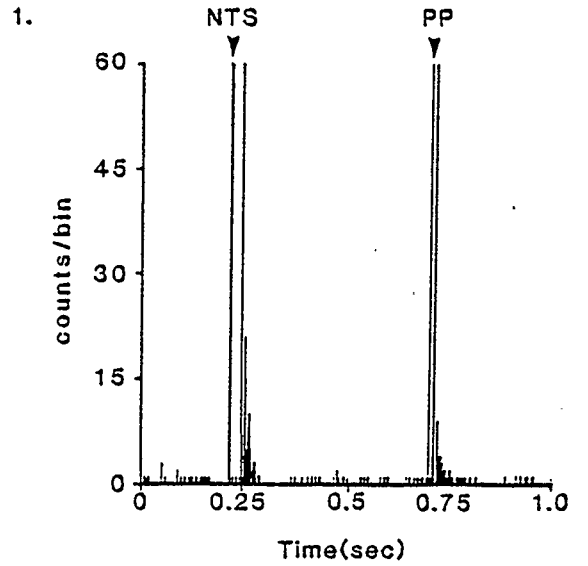


Figure 21. Antidromic criteria for a PVN neuron which was antidromically invaded both by stimulation of the PP and the NTS. Part A shows the three antidromic criteria for PP stimulation, B for NTS stimulation, and Part C gives tracings which show collision of the antidromic potential evoked by PP stimulation with that evoked by stimulation of the NTS. Arrows indicate stimulus artifacts, asterisks spontaneous activity.



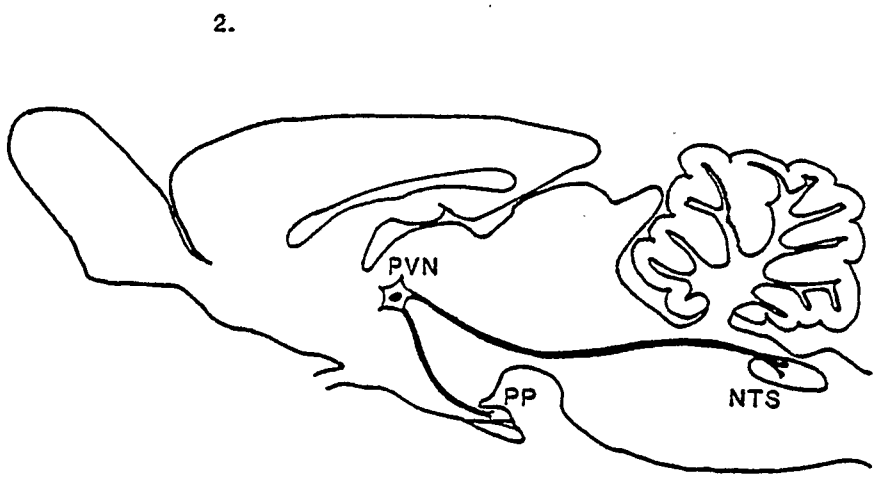


Figure 22. PISH (1) and schematic diagram (2) of the PVN cell identified in Fig.21. The PISH demonstrates that both NTS and PP stimulation are consistently followed by an antidromic potential.

Figure 23. Rate meter records (top) and blood pressure tracings (bottom) of the cell identified in Figs. 21 and 22 which projects simultaneously to the PP and the NTS. Injection of cholecystokinin (CCK) increased cell activity by more than 200% of baseline.

that the two stimuli were applied at least 64 msec apart, both potentials were seen. However, if the interval between stimuli was reduced to less than 63 msec, (i.e. less than the sum of both latencies plus a little for the refractory period of the soma), then the second potential was consistently cancelled. This happened regardless of which stimulus was applied first. The timing of cancellation indicates that the soma either possessed two axons or that the branching point was close to the soma. This neuron was found to reproducibly increase its activity in response to peripheral CCK administration (Fig. 23).

Another cell, illustrated in Fig. 24, also sent axonal projections to both the PP and brain stem. This is an example of a PVN neuron which projects to the VLM and PP simultaneously. However, it was not possible to maintain the activity of this cell sufficiently long enough to examine whether physiological stimuli altered this cells activity. These findings are the first, to my knowledge, of PVN-PP neurons which also projected directly to either the VLM or NTS.

It was consistently observed that neurons which lacked spontaneous activity tended to be unresponsive to physiological manipulations. A typical example of such a cell is shown by Fig. 25.

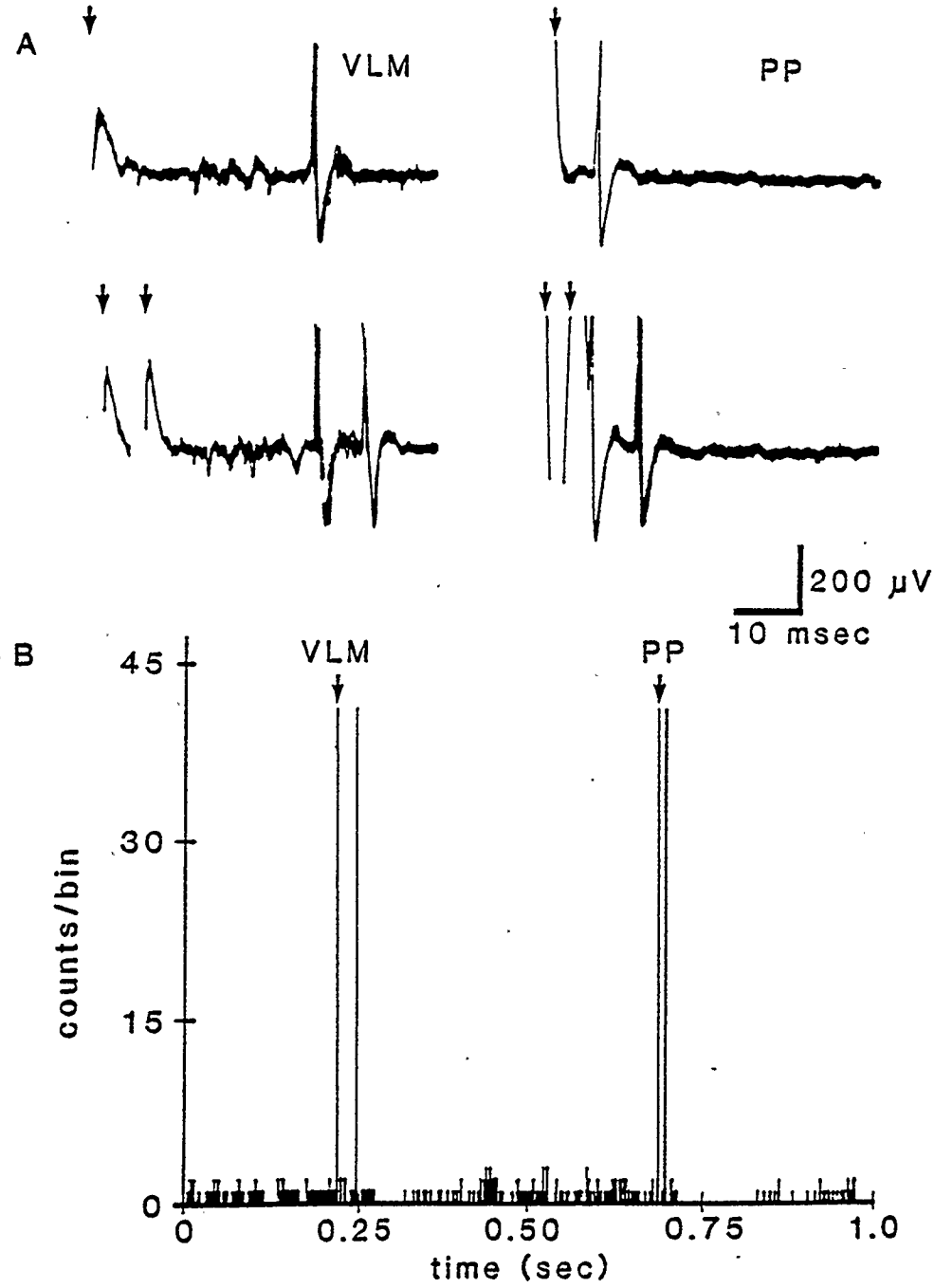


Figure 24. Antidromic criteria (A) and PISH (B) for a PVN neuron which projected simultaneously to both the PP and the VLM. No cellular responses were obtained from this neuron because it was recorded from for only a short period of time.

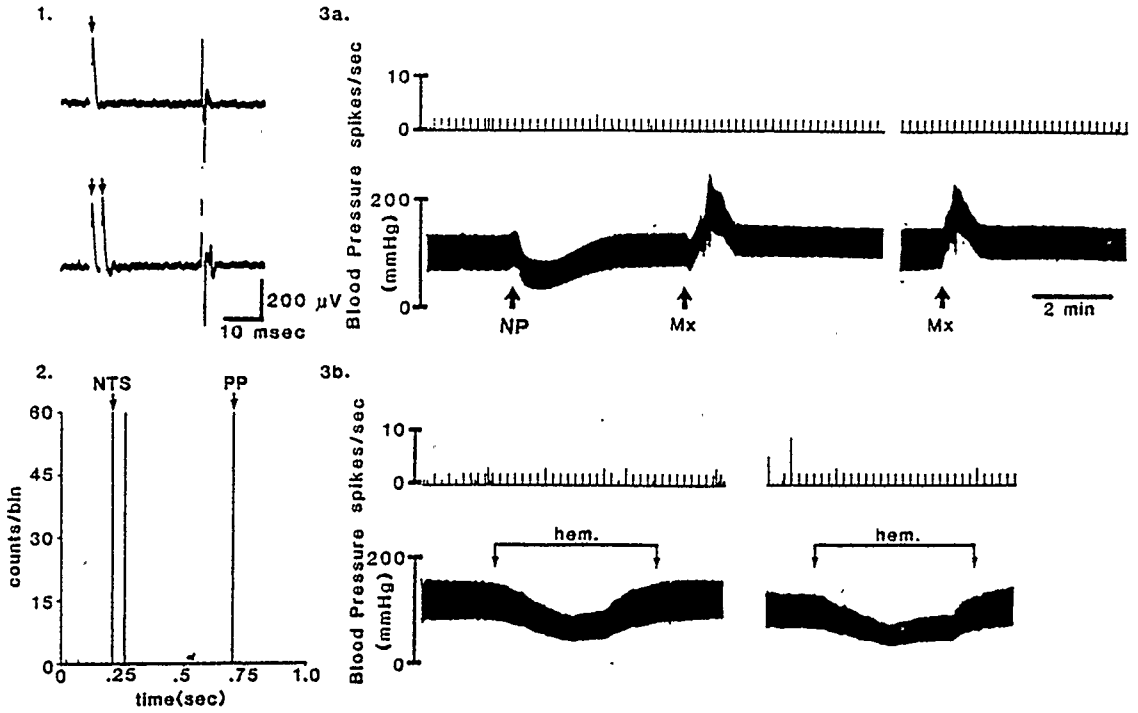
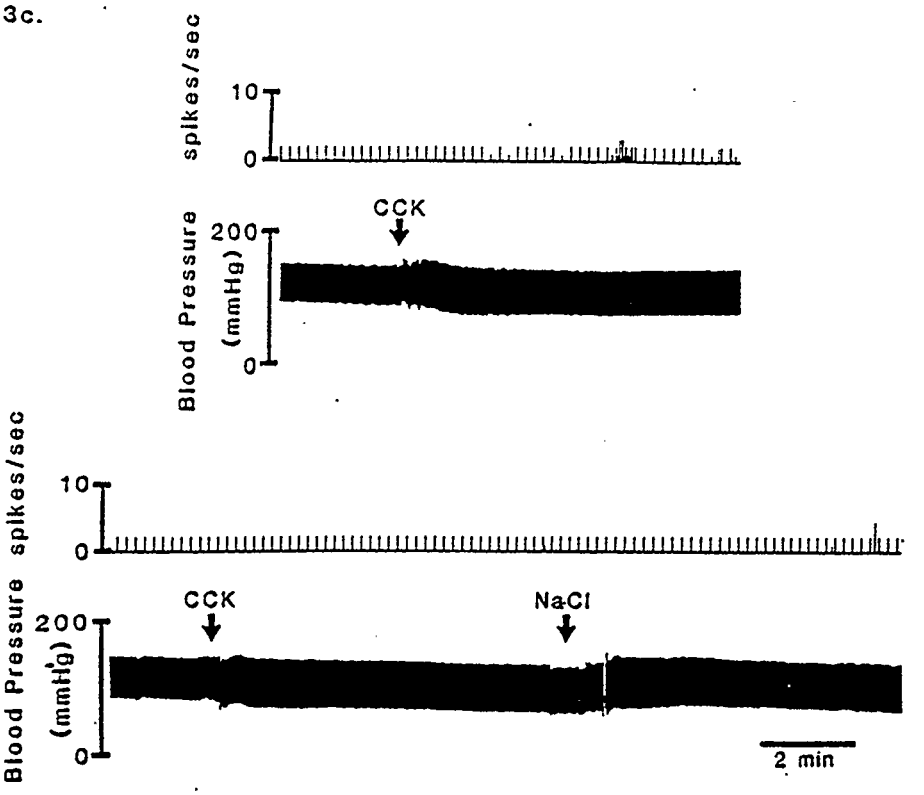


Figure 25. a. Antidromic criteria (1), PISH (2) and rate meter records with blood pressure tracings for a silent PVN-NTS cell (3). The vertical lines on the ratemeter record indicate stimulation of the NTS every 10 seconds. This was done to ensure that the cell was still being recorded from. Any increase in height or frequency of these lines is caused by noise. None of the stimuli stimulated this cell to fire, including increased blood pressure (NP), decreased blood pressure (Mx), or venous hemorrhage of 4.0ml (hem.). The figure on the following page (25. b.) demonstrates that this cell was not responsive to systemic CCK injection or intraperitoneal hypertonic saline injection (NaCl) either.


3c.
spikes/sec
10
0
CCK
Blood Pressure
200
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0
spikes/sec
10
0
CCK
NaCl
Blood Pressure
200
(mmHg)
0
2 min

Figure 25. b.

V. DISCUSSION

A. Analysis of Methods

The present study was performed to determine if the neural projections of the PVN to extrahypothalamic areas of the CNS respond to the same stimuli as neurohypophyseal projections, and if so, do they respond in a similar manner. As a preliminary step, an examination of the viability and usefulness of the animal preparation was carried out. Specifically, experiments were designed to replicate results of previous studies performed on neurohypophyseal neurons of the PVN and/or SON. Consistent to earlier studies (Renaud et al.,1988), some PVN-PP cells were found to fire phasically, however no PVN-brain stem neurons were found to exhibit this firing pattern, supporting previous findings that phasically active cells are only found in magnocellular neurohypophyseal nuclei. This study has also shown that most PVN-PP neurons are sensitive to alterations in blood pressure, plasma osmolality, and/or peripheral CCK. A majority of these cells were found to respond in a manner similar to that reported by others (Renaud et al.,1988), with the cells acting in a reciprocal manner to increased and decreased blood pressure, and being excited by both osmotic stimuli and CCK. Thus, the animal model employed in the present study appears to be appropriate for the purpose of this study.

The techniques employed were useful for identification of neuronal targets from the PVN and for demonstrating what type of stimuli these cells respond to. However, some inherent problems of working with in vivo electrophysiological studies

as this one include the following (Pittman et al.,1987):

(1) The use of anaesthetic is required, and its presence can alter the responses of a neuron to a stimulus. Halothane was used in this study because it is easily administered and monitored, however as with all anaesthetics, it does dampen central function to some extent. Nonetheless, the results of this study show that blood pressure manipulations, hypertonic saline injections, haemorrhage and CCK administration alter neuronal function in a manner similar to that observed in conscious animals (Zerbe et al.,1983; Schadt and Ludbrook,1991; Landgraf et al.,1988). Also, the presence or absence of a response rather than a quantitative response is sought and compared, keeping in mind that the animal was anaesthetized.

(2) Intracellular recording of activity proves difficult due to pulsations caused by blood pressure and respiration, making it virtually impossible to keep the electrode in the cell. However, the use of extracellular recordings are more limited. For example, there is biased sampling aimed toward larger cell bodies, because larger neurons have larger extracellular current fields more easily detected by the recording electrode. Also, using extracellular recording, spontaneously active neurons are more likely to be recorded than are silent neurons. This problem may be overcome by applying stimuli to elicit antidromic potentials in order to identify silent neurons more easily.

(3) Finally, it can be very demanding to alter the environment of the cell and distinguish pre- from postsynaptic effects on that cell. For instance, although a PVN neuron alters its activity in the soma to a specific stimulus, the ultimate consequence

of this response is not known, because activity at the neuronal terminal is not known. There may be pre- or post-synaptic influences on this cell which also regulate the cell's function but remain obscure using these techniques. There might be modulation of neurotransmitter/hormonal release at the axon terminals of this neuron so that one cannot equate the activity of the cell to its ultimate response at the terminal. Also, this neuron might have other projections in addition to those identified, and the manipulation which alters its activity might be important for this other projection. This makes identification of a true response very difficult.

Other obstacles met using extracellular recordings include the difficulty in localizing neuronal groups within the brain. Although stereotaxic coordinates were used to implant electrodes into desired nuclei, there remained variability in the target areas (approximately 0.5mm). However, studies involving stimulation of the NTS and VLM have shown that cellular responses are specific for that area, with noticeably different responses occurring if the electrodes are not near the desired site. However, in this study, the variability of the position of the stimulating electrode was probably acceptable because there was obvious current spread from the stimulating electrode because stimulation of the NTS electrode evoked reflex twitching in the animal. This indicated that the current probably reached motor pathways near the NTS, such as the medial and spinal vestibular paths.

Electrical stimulation is not specific only to neurons which terminate in the nuclei being stimulated, as is glutaminergic stimulation. Instead, all axons in the area, including axons of passage, are stimulated. Thus, one must keep in mind that

elicitation of an antidromic response for an axon terminating in the vicinity of the electrode cannot be differentiated from that evoked from an axon en route to more caudal areas.

In addition, placement of the recording electrode is critical. Most areas of the PVN were sampled in this study as viewed histologically, including both areas containing magnocellular and parvocellular neurons. However, the possibility that certain areas were sampled more often than others and that some PVN subnuclei may have been missed altogether cannot be ruled out. A review of the results found in this study and their significance will be discussed.

B. CARDIOVASCULAR MANIPULATIONS

1) Blood Pressure Alterations

Most neurohypophyseal PVN neurons responded to increased blood pressure by decreasing or arresting cellular activity. In addition, PVN-PP neurons that responded to decreases in blood pressure did so by increasing their activity. This reciprocal response of neuronal activity to blood pressure changes is known to be a typical response of vasopressinergic PVN-PP (Renaud et al.,1988). Thus, the findings in the present study support the utility of the animal model for the purposes of determining responses to blood pressure changes.

In contrast to the above results, very few PVN neurons that projected to brain stem areas (VLM and NTS) were sensitive to blood pressure changes. Further, those cells that did respond were inhibited both by increases and decreases in blood

pressure. Brown and Guyenet (1984) found that the activity of most rostral VLM neurons were inversely correlated with arterial pressure and that changes in activity of these neurons were closely coupled in time to arterial pressure changes. In contrast to this finding, however, it was observed in this study that PVN neurons projecting to the VLM displayed decreased cell activity in response to either an increase or decrease in blood pressure. This suggests that projections could be onto two different classes of neurons in the VLM. In support of this, two different types of cardiovascular neurons, being positively and negatively correlated with blood pressure changes, have been identified within the ventrolateral medulla, both which receive innervation from the PVN (Brown and Guyenet,1984; Maeda et al.,1988). In fact, 45% of all synapses in the VLM contain GABA, and thus the activation of these afferents could cause increased release of GABA, and thus an indirect inhibitory effect. It is also possible that descending projections from the PVN contain both excitatory and inhibitory transmitters; activation of an excitatory pathway could have the same postsynaptic effect as a reduction in activity of an inhibitory pathway.

Finally, the demonstration of several different responses of PVN neurons to the same stimulus underlines the heterogeneity of cellular responses within the PVN. For example, the typical recorded result of a phasic neuron to increased blood pressure is that cellular activity stops completely during the period that blood pressure is increased. This result was also found in this study, however, one phasic neurohypophyseal PVN neuron (Fig. 7) kept firing even when blood pressure was

raised to 185mmHg. The bursting intervals, however, did shorten and interburst intervals were lengthened. This result becomes important when one considers the evidence suggesting that the relationship between mean firing frequency and amount of hormone released per impulse is not linear, and that the rat PP kept in vitro releases more hormone per impulse the shorter the interval between action potentials. (Dreifuss et al.,1971). Thus, although the cell does not decrease firing altogether, it may not have to in order to stop or decrease V release into the systemic circulation, as will be described later. Variability of responses from one cell to another was also found to occur following other stimuli, such as CCK administration and haemorrhage. These findings support the view that the PVN is a heterogeneous nucleus made of different cell types with diverse functions.

2) Changes in Blood Volume

The cellular responses to decreased blood volume via haemorrhage in this study varied from cell to cell. Approximately one-third of all PVN-PP neurons recorded from during haemorrhage of the animal responded to this manipulation; half decreased their activity, whereas the other half were excited. However, haemorrhage was found not to be a potent stimulus for PVN-brain stem neurons. Previous studies predict that cells should increase their firing and thus release more hormone/neurotransmitter to cause a pressor effect in the event of blood loss due to haemorrhage. This would occur in an attempt to maintain blood pressure at a normal level (Johnson et al.,1988).

A possible explanation for the finding that some PVN neurohypophyseal cells and most brain stem projecting PVN neurons were not responsive to haemorrhage may be that the duration or magnitude of the haemorrhage may not have been great enough to activate AVP release to cause vasoconstriction. In fact, Schadt and Hasser (1991) reported that AVP release into the circulation was not important in blood pressure maintenance either early in haemorrhage or at the onset of hypotension. Support for this came from Schadt and Ludbrook (1991) who described two phases of haemorrhage. The first stage involves sympathetic stimulation to maintain arterial blood pressure, but does not involve V release. However, once blood volume has fallen by a critical amount, or the haemorrhage has lasted for a certain time, the second phase develops abruptly. This phase is characterized by withdrawal of sympathetic vasoconstrictor drive, bradycardia, increased release of V, and an acute fall in arterial pressure.

Neurons that were inhibited by haemorrhage may have been under some type of inhibitory influence. It is known that the increase in V release for blood volume recovery is inhibited by opioid peptides during acute haemorrhagic hypotension (Schadt and Hasser,1991). This inhibition may have caused the decrease in PVN cell firing in response to haemorrhage. Inhibition of PVN neurons might explain the finding in this study of several neurons lacking spontaneous activity and insensitive to any physiological manipulation. These neurons may also have been under opioid inhibition.

3) Ionic Manipulations

More than half of the neurohypophyseal PVN projecting neurons tested for a response to hypertonic saline injection responded by increasing their activity. In contrast, no PVN neurons projecting to brain stem areas were sensitive to this manipulation. However, very few PVN brain stem neurons were tested for this stimulus and thus no definite conclusion can be drawn from these results.

Intraperitoneal injection of hypertonic saline increases plasma osmolality as well as plasma AVP and OT (Landgraf et al.,1988; Brimble et al.,1978) via both central and peripheral pathways (Landgraf et al.,1988). This increase in osmolality has been found to occur gradually over a time course of 15-30 minutes (Dunn et al.,1973). Thus, one would expect to see increased firing of PVN neurons after at least 10-15 (ex.Fig. 15). However, cells were found whose activity was increased immediately after intraperitoneal injection of hypertonic saline. This immediate activation may be due to peripheral osmosensitive receptors with neural projections directly to the PVN. However, an alternative explanation may be that this activation is due to the pain involved with the intraperitoneal injection of the hypertonic saline. This possibility has previously been suggested by Landgraf et al. (1988), and is supported by the observation that nociceptive information is relayed to the NTS and PVN (Yirmiya et al.,1990).

4) Angiotensin II Effects

Intravenous injection of angiotensin II (AG) was found to activate PVN-PP neurons. However, due to the small number of cells tested for this stimulus, no conclusions can be drawn. However, this preliminary result does support previous findings that angiotensin II stimulates both OT and AVP hypothalamic neurons (Akaishi et al.,1980).

C. Cholecystokinin Injection

Peripheral administration of CCK has been found to increase circulating levels of OT, but not AVP, in rats. This is probably due to stimulation of magnocellular OT neurons of the supraoptic nucleus (SON; Renaud et al.,1987) and PVN (McCann et al.,1989). Results from this study support this finding since several neurohypophyseal PVN neurons were excited by intravenous CCK administration. In contrast to this, few PVN cells projecting to the NTS responded to peripheral CCK and none of the neurons that projected to the VLM responded. These results do not support the findings of Verbalis et al. (1991) that systemic CCK administration activates c-fos expression in parvocellular OT and corticotropin releasing hormone (CRH) neurons, nor their hypothesis that systemic CCK causes secretion of OT and CRH within the brain at terminal fields of these neurons. The proto oncogene c-fos can be rapidly induced in discrete neuronal populations in response to a variety of stimuli, used as a marker for transcriptional stimuli in these areas. For example, Verbalis et al.(1991) found that the pattern of c-fos expression

in hypothalamic magnocellular neurons following systemic CCK administration mirrors the neurosecretory response of these neurons.

Receptors for CCK have been located in the vagus nerve, NTS and PVN (Silver and Morley,1991). Peripheral CCK usually excites PVN neurons, however, results from this study show that some neurons projecting to the PP and NTS are inhibited by systemic CCK. In fact, Renaud et al. (1987) also observed that a few putative AVP-secreting cells in the SON exhibited depressed activity in response to systemic CCK. A possible mechanism for this inhibition is that peripheral CCK activates vagal afferents to the NTS. In fact, CCK has been found to excite NTS neurons (Silver and Morley,1991) which in turn relays this information to the PVN (Renaud et al.,1987). Due to the fact that the PVN fires reciprocally in response to NTS innervation, increased activity in the NTS would result in decreased PVN activity through this circuitry. Therefore, increased activity of NTS neurons by direct CCK excitation could result in the inhibition of PVN neurons seen in this study and that by Renaud et al.(1987).

The stimulation of OT release, such as by CCK stimulation, has been implicated in several functions. These include milk ejection in lactating females (Wakerley and Lincoln,1971), uterine contraction for parturition (reviewed by Richard et al.,1991), regulation of plasma osmolality (Brimble et al.,1978), gastric function (Orosco et al.,1990; Leibowitz,1978) and nociception (Lang et al.,1983; Yirmiya et al.,1990). Specific CCK pathways are activated in response to peripheral stimuli associated with feeding. This probably occurs by stimulation of receptors

located on afferent fibers of the gastric vagus (Zorbin et al.,1981) which enhance sensory input from the stomach to the NTS (Raybould et al.,1985). Accordingly, gastric vagotomy has been reported to abolish the CCK-induced stimulation of OT secretion and inhibition for food intake and gastric emptying (McCann et al.,1989). In agreement with this, Renaud et al. (1987) found that gastric distention also stimulates OT release from the SON. This evidence indicates a sensitive neural link between the stomach and the hypothalamus.

Intravenous injection of CCK often resulted in a short-lasting alteration of blood pressure in this study (see Figs.12,15 & 16). This transient change in blood pressure may be due to residual methoxamine in the intravenous line, or it may be due to a direct activation of vagal afferents, since there are CCK receptors on the vagus (Verbalis et al,1986) that reach the NTS where CCK might act as a neurotransmitter (Silver and Morley,1991). This vagal activation could result in the effect of CCK seen on blood pressure. No effect of CCK administration on blood pressure has previously been reported to my knowledge. For example, Renaud et al. (1987) performed similar experiments as the present study, but he did not report or show any effect of intravenous CCK injection on blood pressure. One reason for this may be that the preparation used in the present study was more representative of the physiology of the conscious animal. For instance, mean arterial pressure of the rats used by Renaud was approximately 60 mmHg, in contrast to 90 mmHg as in the present study. He also recorded only mean arterial pressure rather than systolic and diastolic arterial pressures as in the present study. This may hide any small

alterations in blood pressure occurring due to CCK as shown by the present results.

The responses of cellular activity to CCK injection were not caused by the very small alterations in blood pressure observed in this study because the blood pressure changes were too transient and short lasting compared to the cell activity response. The cell activity was altered for several minutes after injection of CCK, whereas the blood pressure was altered for only a few seconds. Thus, the cellular activity was not correlated with the blood pressure effect of CCK.

D. Central versus Neurohypophyseal PVN Projections

Results presented in the present study suggest that although cells projecting from the PVN to the PP are involved with cardiovascular regulation, including regulation of blood pressure, volume and osmolality, as well as CCK responses, proportionately fewer neurons projecting to the brain stem seem involved. This finding could be a consequence of having sampled inadequate numbers of the right population of descending neurons. However, the populations of descending neurons (to NTS and VLM) sampled were greater in number than those projecting to the PP. Therefore, there should have been equal opportunities to observe such cells. If indeed the results are representative of all descending parvocellular neurons, the functional consequence of this could be twofold: few PVN neurons descend to the brain stem because this pathway is of little importance for blood pressure maintenance, or these projections might be involved in some critical cardiovascular control in which a few neurons perform some crucial and extensive function.

Axons deriving from PVN neurons project to and terminate in both vascular and neural target areas (Sofroniew ,1985). There are two vascular targets that PVN neurons innervate: the PP and the external zone of the median eminence which contains hypophysial portal vessels. However, neural target areas include all of the PVN projections to CNS areas, including the intermediolateral cell column of the spinal cord (IML), NTS, and VLM. There is evidence that the nature of the terminals found in these two types of targets may be different. Immunohistochemically identified axons of PVN neurons grown in hypothalamic cultures exhibit both collaterals in passing and extensive terminal arborization (Sofroniew et al.,1986). This differs from vascular targets, which are reticular in appearance. Thus, individual PVN neurons which make neural connections may be responsible for a large number of terminals, which are sometimes diffusely spread over a large area. Therefore, in contrast to vascular PVN projections, few neurons from the PVN innervating neural areas may give rise to fairly extensive innervation (Sofroniew,1985).

Also, Sofroniew and Glasman (1981) found that neurons in the PVN that give rise to 2 axon-like processes may be more prevalent than previously thought. In fact, approximately 1.5% of all neurons recorded from in this study projected to both the PP and to the brain stem, which is comparable to the 0.8% found by Yamashita et al. (1984) projecting both to the PP and IML.

Dual projections suggest there is coordinated activity of this neuron at two different terminals, but they also suggest that one cell could affect many different

neurons. This may be important due to the differences in numbers of projections to the brain stem or PP, since Swanson and Kuypers (1980) discovered that a significant proportion (10-15%) of the neurons with descending projections send divergent axon collaterals to both the medulla and spinal cord directly to activate the autonomic nervous system. Thus the result of stimulation of one centrally projecting PVN neuron may be more potent than projections to the PP because these neurons may activate more than one area due to axon collaterals.

E. Alternative Functions of the PVN

In addition to V and OT in magnocellular SON and PVN neurons, all or some have been reported to contain immunoreactivity for a large number of peptides and other substances including somatostatin, enkephalin, AG, renin, glucagon, corticotropin releasing factor, CCK, dynorphin and tyrosine hydroxylase (Sofroniew et al.,1985). Thus, neural projections from the PVN not shown to respond to the manipulations employed in the present study (blood pressure, volume, osmolality, and CCK) could be involved in functions which were not tested for in the present study. Possible alternate functions include nociception (Yirmiya et al.,1990; Lang et al.,1983), milk ejection and uterine contraction (Wakerley and Lincoln,1973), gastric function (McCann et al.,1989; Leibowitz,1978; Orosco,1990; Olson,1991), and the generation of circadian rhythms (Tribollet and Dreifuss,1981).

F. Conclusions and Future Work

In conclusion, results of this study support a role for neurohypophyseal neurons of the PVN in cardiovascular regulation as well as for a small population of central PVN neurons. However, the numbers of these two groups involved in cardiovascular regulation differ, with very few of the centrally projecting neurons being involved. However, the few PVN neurons found to project to both the PP and brain stem areas, indicate that some central and peripheral PVN projections could act in a coordinated manner to regulate the cardiovascular system.

Future studies to determine the function of the central projections of the PVN might involve the use of c-fos, for example, to identify neuronal populations which are activated by specific stimuli such as blood pressure changes. In this way, the stimuli which activate central PVN projections could be found. Also, more manipulations could be carried out in an attempt to identify stimuli to which centrally projecting PVN neurons respond. One could set up a study in which suckling pups were present, because this has been found to be a potent stimulus for OT stimulation by the neurohypophysis (Lawrence and Pittman,1985). Also, the opioid antagonist, naloxone, could be used to examine how centrally projecting neurons respond to stimuli when they are released from the opioid inhibition they normally experience.

VI. LIST OF REFERENCES

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