The University of Calgary

Electrophysiological Studies on Vasopressin Mediated Neurotransmission

by

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A Thesis

Submitted to the Faculty of Graduate Studies

in partial fulfillment of the requirements

for the degree of Master of Science

Department of Medical Physiology

Calgary, Alberta

December, 1985

ISBN Ø-315-29932-Ø

THE UNIVERSITY OF CALGARY

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Electrophysiological studies on vasopressin mediated neurotransmission", submitted by Jean E. Disturnal in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

Electrophysiological investigations of the ventral septal area of the brain were made to further define the antipyretic actions of arginine vasopressin and to provide further evidence for arginine vasopressin as a central neurotransmitter.

Single unit extracellular recordings were used to demonstrate afferent projections from the paraventricular nucleus (PVN) and bed nucleus of the stria terminalis (BST). These nuclei have previously been shown to contain immunoreactive vasopressin containing cell bodies and were therefore identified as the potential sources of vasopressin in the ventral septal area.

In vivo microelectrode recordings also revealed a large proportion of identified neurons in the ventral septal area responsive to peripheral thermal stimulation of the scrotal skin. This observation provided further evidence to implicate the ventral septal area as a component of thermoregulatory pathways in the brain. The 3.8:1 ratio of warm to cold responsive neurons studied also suggested that the function of this area may involve heat loss mechanisms, supporting a role for the ventral septal area in antipyresis.

Evidence that vasopressin may be associated with the thermoregulatory processes in the ventral septal area was provided by the observation that thermoresponsive neurons in this area received input from the PVN which was primarily inhibitory and the BST which appeared to preferentially inhibit warm responsive and excite cold responsive ventral septal neurons.

Iontophoretic application of vasopressin on ventral septal neurons caused a reduction in the excitatory response of these neurons

to glutamate. PVN and BST inhibitory afferent projections were also observed for these vasopressin responsive neurons. This evidence suggested that vasopressin acts in the ventral septal area to modulate the neuronal response to glutamate.

Of the thermoresponsive neurons tested, 85% were also found to be responsive to vasopressin application and received afferent projections from the PVN and BST. This evidence suggested that endogenous vasopressin, possibly arising from the PVN and BST, acts on thermoresponsive neurons in the ventral septal area as a neuromodulator of glutamate evoked neuronal activity.

ACKNOWLEDGEMENTS

My sincere thanks go to Dr. Q.J. Pittman and Dr. W.L. Veale for their excellent supervision of my graduate work and for their encouragement, support and patience during the time I spent in the lab. Thanks also to Dr. B. MacVicar for his helpful advice and stimulating discussions and for serving on my supervisory committee. Thanks to Dr. B. Bland for reading this thesis and serving on my examining committee. I would also like to thank L. Franklin for her technical assistance and all the members of the two laboratories for their assistance and encouragement whenever it was needed. Thanks to H. Toft for her patience in typing this thesis. I acknowledge financial support from the Alberta Heritage Foundation for Medical Research.

I would also like to extend special thanks to my parents for their continuing support, understanding and encouragement.

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i) Historical Aspects of Thermoregulation

The regulation of body temperature within physiological limits is a homeostatic process essential to the survival of a number of animal species, so essential in fact, that in times of physiological stress temperature regulation is given priority over other regulated systems. This fundamental relationship between heat and life was recognized even in ancient times when Greek philosophers proposed that the body was composed of four essential elements: earth, air, fire and water. Body heat was thought to derive from a central fire which was located in the heart and respirations were proposed as the cooling mechanism (Lomax, 1979). These concepts persisted until the 15th century when the use of a primitive thermometer invented by Giovanni Borelli failed to show that the heat intensity of the heart was greater than that of other organs in the body (cf Fulton & Wilson, 1979). The theory of a central fire as the heat source was then replaced by the theory that body heat derived from friction of the circulating blood against vessel walls. Although this was consistent with the observations of that time, many scientists were skeptical that the large amount of heat in the body could be attributed to friction of circulating blood cells.

In 1780 Lavoisier was able to integrate the recent thermodynamic advancements of this time and hypothesized that the major part of animal heat originated from the combustion of oxygen with organic

substances in the animal's body. At about the same time, the concept of a balance between heat production and heat loss processes in the body was beginning to be appreciated. Blagden and others (1775) performed a number of experiments in which they observed that heat loss in man was accomplished by the evaporation of sweat from the skin in a warm environment. Further, in 1778 John Hunter observed that in the cold certain animals increased their heat production in order to maintain a constant body temperature while other species showed a reduction of body temperature to that approximating the ambient environment. This provided the first evidence which clearly defined the differences between homeothermy and poikilothermy. It then became an accepted concept that separate heat production and heat loss mechanisms existed which could explain the uniformity of body temperature in healthy, warm blooded animals. However, the way in which this constant temperature was maintained remained elusive.

Currie (1798) has been attributed to be the first to allude to temperature as a regulated function, but it was Brodie in 1811 who performed the classical experiments which provided evidence for body temperature being dependent not only on chemical reactions but nervous system regulation as well. He showed that when the neck vessels of an animal were ligated and the animal artificially ventilated, body temperature fell despite the fact that oxygenated blood continued to perfuse the tissues. Chossat (1820) then showed that transection of the spinal cord caused cooling in the dog and that the higher the level of transection, the greater the cooling. Tschischichin (1866) made similar observations and established that these results could be attributed to the influence of the brain on the spinal cord.

In 1876 Claude Bernard introduced his theory of homeostasis in which he recognized the body's ability to regulate the internal environment or "milieu interieur". With respect to temperature regulation, Bernard agreed with the hypothesis of Lavoisier (1780) that body heat derived from the oxidation of organic substances and provided further evidence for metabolic processes as the source of an animal's heat. He also proposed that heat loss was accomplished through nervous regulation of blood flow to the surface of the body. The temperature around which the body was regulated was suggested to be determined by a dynamic balance between the heat production and heat loss mechanisms.

Following the acceptance of central nervous system involvement in the regulation of body temperature, attention turned to locating the thermoregulatory centres in the brain. Ott (1887) indicated that lesions of "the anterior inner end of the optic thalami . . . causes an increase of temperature due to increased heat production" (pg 154). Barbour (1912) showed that heat applied to the corpus striatum caused body temperature to drop while cold applied to the same area resulted in an increased body temperature.

The hypothalamus was identified a major thermoregulatory centre in the brain when it was found that discrete lesions of select regions of the hypothalamus resulted in impaired thermoregulatory ability in a number of homeothermic animals. Lesions of the posterior and anterior hypothalamus were shown to cause a loss of heat production and heat dissipation mechanisms in both hot and cold environments (Clark et al, 1939; Anderson et al, 1965; Squires and Jacobson, 1968; Carlisle, 1969, Satinoff and Rutstein, 1970). Lateral hypothalamic lesions

impaired behavioral thermoregulatory responses (Satinoff and Shan, 1971) while ablation of the preoptic anterior hypothalamus was shown to interfere selectively with autonomic thermoregulatory mechanisms (Carlisle, 1969; Satinoff and Rutstein, 1970). The preoptic anterior hypothalamus was further proposed as the primary thermoregulatory structure in the brain when it was demonstrated that both local temperature changes (Beaton et al, 1941; Calvert and Findlay, 1975) and electrical stimulation (Anderson et al, 1956; Anderson, 1957; Hemingway et al, 1954) of this area resulted in altered thermoregulation.

ii) Thermoreception and Temperature Regulation

It is now known that control of thermoregulatory functions by the central nervous system is based on the ability of the brain to receive and integrate thermal information from a variety of sources prior to activation of appropriate effector systems. Neuronal structures with a specific sensitivity for temperature (thermoreceptors) were first described in the tongue of the cat by Zotterman (1935, 1936). Since that time, thermoreceptors have been characterized in the periphery, deep body tissues and brain of a variety of animals. Although all biological processes, including neurophysiological ones (ie. the maintenance of resting potential), are inherently temperature dependent, thermoreceptors are argued to be specialized structures which are also essential components of thermoregulatory systems.

A large portion of the literature on thermoreceptors has been devoted to the analysis of the individual firing characteristics of

these neuronal structures. As a result of this work, four fundamental properties of all thermoreceptors have emerged:

1) Thermoreceptors show a static discharge at a constant temperature.

2) Thermoreceptors show a dynamic response to temperature changes. Warm receptors increase firing with an increased temperature (a positive temperature coefficient) and cold receptors increase firing with a decrease in temperature (a negative temperature coefficient).

3) Thermoreceptors are not excited by mechanical stimulation.

4) Thermoreceptor temperature dependent activity in the periphery occurs in the nonpainful temperature range.

(Hensel, 1981)

a) Cutaneous Thermoreceptors

Specific warm and cold cutaneous thermoreceptors demonstrate different morphologies and firing characteristics. Warm receptors are thought to exist as free nerve endings below the epidermis which project to the central nervous system primarily as thinly myelinated Ad fibres (Hensel, 1974). Electron microscopy combined with electrophysiological studies has identified cold receptors in the cat's nose as free nerve endings which arise from thin myelinated axons which divide into several nonmyelinated terminals within the stratum papillare. The terminal axons have been shown to be associated with Schwann cells as far as the epidermal basal lamina with the receptive ending penetrating the epidermis. The importance of these anatomical differences between warm and cold receptors to their function remains to be be determined.

The study of the firing characteristics of warm and cold receptors has not revealed any consistencies which may be used to electrophysiologically distinguish between them. The temperature range over which thermoreceptors are most active is an individual characteristic of each neuron and only determined by the direction of change and not the absolute temperature. As well, different firing characteristics can be elicited by altering the rate of temperature change. However, it has occasionally been shown that cold receptors display bursting firing patterns which have not been described for warm receptors (Hensel, 1981). Further warm receptor spontaneous discharge is enhanced and cold receptor discharge inhibited by local injection of calcium (Hensel and Schafer, 1974). Although this phenomena has yet to be clarified, it may lead to a mechanism to explain how warm and cold receptors demonstrate their different properties.

b) Deep body thermoreceptors

The existence of thermoreceptors in the deeper tissues of the body has long been postulated. Evidence for thermoreceptive stuctures in the circulatory system has been obtained from recordings of afferent nerve fibres serving intact isolated veins (Minot-Sorokhtina, 1972; cf Hensel, 1974) and from splanchnic nerve recordings during intraabdominal heating. The neuronal activity of the intraabdominal thermoreceptors during local heating were found to demonstrate similar characteristics to spinal thermoreceptors during heating of the spinal cord (Riedel et al, 1973). Further evidence for deep tissue thermoreceptors and their involvement in thermoregulation is provided

by experiments showing that panting can also be elicited by intraabdominal heating (Ingram and Legge, 1972).

c) Central thermosensitivity

Central thermoreceptive structures were postulated when a number of investigators reported that thermoregulatory responses could be elicited by using brain implanted thermodes to change the temperature of the brain at discrete sites (cf Bligh, 1973). In particular, the anterior hypothalamic area was shown to be especially sensitive to these local temperature changes. Heating the preoptic anterior hypothalamus (POAH) in a thermally neutral environment activated vasodilation, salivation and sweating and reduced cold induced vasoconstriction and shivering. Cooling the POAH region elicited shivering, increased O2 consumption, blood flow decreases in the skin and increased cardiac output which was similar to that of an animal placed in a cold environment (Hales et al, 1976, 1977; cf Hensel, 1981).

Based on this indirect evidence for central thermoreceptors, electrophysiological experiments were conducted which characterized both warm sensitive or cold sensitive neurons that responded to temperature changes of the hypothalamus which produced the previously observed thermoregulatory effects (Hellon, 1967; Reaves and Heath, 1975; Mercer et al, 1978). An extensive analysis of the data now indicates that 20-40% of the neurons in the ventral septum, preoptic area and anterior hypothalamus respond with positive or negative temperature coefficients to changes in hypothalamic temperature (cf Eisenman, 1982). Although the posterior hypothalamus appears to be less thermosensitive than the POAH (Hayward, 1977), thermosensitive

neurons (neurons which respond to local temperature changes) have also been reported in this region (Cabanac et al, 1968). The potential involvement of these neurons in the thermoregulatory system is not clear. In contrast to cutaneous thermoreceptors, the central thermosensitive neurons have not been morphologically studied. This makes it difficult to determine whether a particular neuron under study detects local temperature changes itself or is an interneuron driven by a central thermodetector. Contributing to this problem is the anatomical complexity of the hypothalamus which lacks isolated pathways or functional centers. Therefore, there is no certainty that thermosensitive neurons in the hypothalamus play a role in the transmission or detection of thermal information. Nonetheless it is an accepted presumption that thermosensitive neurons observed in a brain region known to elicit appropriate thermoregulatory responses to local thermal and electrical stimulation are indeed components of thermoregulatory pathways.

Extrahypothalamic structures also contain thermosensitive neurons which are implicated in thermoregulatory pathways. Local heating and cooling of the medulla oblongata (Chai and Lin, 1972; Inoue and Murakami, 1976; Lee and Chai, 1976) and spinal cord (Simon et al, 1974; Jessen, 1971) has been shown to elicit appropriate thermoregulatory responses and to facilitate the characterization of warm and cold sensitive neurons. While thermosensitive neurons have been identified by altering the local temperature of a number of other central areas, their involvement in thermoregulatory pathways awaits further lesion and electrical stimulation studies.

d) Thermal afferent pathway

Single unit electrophysiological studies have been used to trace the thermal afferent pathway from the peripheral thermoreceptors to higher order neurons in the brain. Quantitative relationships between thermal stimuli and neural response are difficult to establish since most central neurons which respond to peripheral temperature (thermoresponsive cells) have been shown to converge with other individual cutaneous thermoreceptors as well as with mechanoreceptors and nociceptors in their afferent projections to the brain (Burton, 1975; Iggo and Ramsey, 1976; Zimmerman, 1977). Allowing for these limitations, studies on the thermal afferent pathway have often used the scrotum as a site for cutaneous thermal stimulation. This has been a popular preparation since not only can thermal stimulation of the scrotum be precisely controlled, but scrotal skin has been shown to contain a high density of cutaneous thermoreceptors (Hellon 1975), a large number of neurons in the brain respond to changes in scrotal temperature (Nakayama et al, 1979) and the scrotal skin appears to provide a powerful drive to thermoregulatory circuits (Waites, 1962; Ingram and Legge, 1972).

Thermal information from the cutaneous thermodetectors is transmitted to the brain via primary afferent projections to the dorsal horn of the spinal cord where, through synapses with secondary neurons in the dorsal root ganglia, it ascends in the ventrolateral quadrants of the spinothalamic tract to the ventrobasal thalamus. Both crossed and uncrossed projections have been described (Hellon, 1975). It has recently been reported that direct spinothalamic projections are not used but that thermal information relays in the

nucleus raphe magnus of the midbrain before projecting to the thalamic nuclei (Gottschlich and Werner, 1985; Gottschlich et al, 1984). Above the level of the thalamus thermoregulatory pathways are less clear. Thermoresponsive neurons have been identified in a number of brain areas but because the activity patterns of the peripheral thermoreceptors and the cerebral thermoresponsive neurons they presumably drive can be very different, it is not known whether these units are components of ascending, integrative or descending pathways.

e) Central integration of thermal information

It is well established that thermoregulatory responses, whether autonomic or behavioral, can be induced by temperature stimulation in various locations of the body. It is equally apparent that the observed response is a result of the integration of a combination of thermal inputs. Therefore, the major question is not whether integration occurs, but rather where and how.

The hypothalamus appears to be the centre of integration of a number of autonomic responses. The POAH has further been identified as the primary structure involved in the control of autonomic thermoregulatory responses. Evidence for the integration of thermal signals in the POAH has been provided by experiments which show that changes in skin temperature modify the thermoregulatory responses to warming and cooling of the anterior hypothalamus (cf Bligh, 1973). Increased skin temperature has been shown to inhibit peripheral blood flow responses induced by hypothalamic cooling in conscious animals (Knudt et al, 1957; Ingram and Legge, 1972) while the activation of peripheral cold thermoreceptors inhibits heat dissipation mechanisms elicited by central warming (Cabanac, 1975).

This interaction between hypothalamic temperature and skin temperature has also been shown in reverse. As demonstrated by Knudt et al (1957), an increase in hypothalamic temperature could be induced by cooling the paws of cats thereby showing that cold defense reactions activated by cooling of the skin oppose the activity of warm receptors in the hypothalamus.

Integration of skin and hypothalamic thermal information is also revealed by electrophysiological studies. Wit and Wang (1968a) originally reported that hypothalamic thermosensitive neurons could also be driven by changes in skin temperature. The convergence of peripheral and POAH temperature input has since been confirmed in a number of electrophysiological reports which indicate that between 40-75% of thermosensitive neurons in the anterior hypothalamus were affected by changes in both local and peripheral temperature (cf Eisenman, 1982). A number of mathematical models for thermal integration have been proposed based on this data, however it is still unclear whether the interactions between skin and central thermal drives in the anterior hypothalamus are additive or multiplicative.

The posterior hypothalamus has also been implicated in the central integration of thermal signals. Interactions between neurons responsive to thermal stimulation of the posterior hypothalamus, POAH and spinal cord have all been shown to occur in the posterior hypothalamus (Wunnenberg and Hardy, 1972; Dymnikova, 1973; Nutik, 1973).

Relatively little is known about the precise pathways and mechanism of integration of thermal signals in the central nervous system. Brain regions from the septal-preoptic area rostrally to the

caudal posterior hypothalamus have all been shown to participate in the sensing of temperature, the integration of afferent pathways and the organization of efferent pathways. The development of mathematical models has aided in the understanding of integrative mechanisms, however it is still a debate whether the processes of heat production and heat dissipation are coordinated in one area or located in separate brain regions.

B. Fever

i) Introduction

Fever is a pathological condition in which the body temperature is elevated up to 5o C above normal and maintained for a variable length of time. Hippocrates recognized fever as an important symptom of illness and noticed that the rise in body temperature was often associated with various forms of inflammation (Adams, 1939). In 1875 Leibermeister postulated that body temperature was regulated by the central nervous system at a higher level during fever. It is now recognized that during fever the body increases its temperature through actively controlled processes. The mechanisms used for heat gain by the body are dependent on the ambient temperature. In a warm environment, the increased temperature in fever is accomplished by an inhibition of heat dissipation mechanisms. In the cold, the same febrile temperture is attained primarily through the activation of heat production mechanisms (Cooper, 1979a; Cooper and Veale, 1974; Grant, 1949). It is the regulated rise in body temperature during fever which distinguishes this state from that of hyperthermia in which a nonregulated, passive increase in body temperature occurs.

The central mechanisms used to determine and maintain a febrile temperature are not clearly understood. A set point mechanism has been proposed which suggests that body temperature is regulated around an arbitrary set point temperature. In fever, the set point is raised to a higher level and the increased body temperature observed is a result of the activation of thermoregulatory mechanisms to attain the higher set point temperature (Bligh, 1976). In support of this theory is the observation that during fever, the body actively defends the febrile temperature against hot or cold thermal challenges (Macpherson, 1959; Cooper et al, 1965). Myers and Veale (1971) further investigated the central mechanism for the set point theory. Infusion of excess Na^+ in the posterior hypothalamus of the cat was observed to evoke a marked increase in body temperature accompanied by shivering, vasoconstriction and piloerection. When the same site was perfused with Ca^{++}, body temperature decreased. During fever, the ratio of radioactive labelled endogenous stores of Na^+ and Ca^{++} also shifted as body temperature was increased. Based on these results, they proposed that the set point may be established by a dynamic balance between sodium and calcium ions in the posterior hypothalamus.

In contrast to the theory of an elevated set point mechanism of fever are electrophysiological studies which indicate that rather than the expected shift in the temperature at which warm sensitive neurons are activated, their thermosensitivity decreases following pyrogen treatment (Cabanac et al, 1968; Eisenman, 1969; Eisenman, 1974; Wit and Wang, 1968b). The functional significance of this observation is not clear since other studies of thermoregulation during fever do not

show any decrease in regulatory precision or capacity (Macpherson, 1959; Cooper et al, 1964; Cranston et al, 1976).

ii) Pyrogens

a) Exogenous pyrogens

A number of substances are known to initiate the febrile process including endotoxins, gram positive bacteria and viruses. The most potent of exogenous pyrogens are endotoxins which derive from the cell wall of gram negative bacteria. This bacterial pyrogen is a large 1-2 million molecular weight molecule with three identifiable components: a polysaccharide core, an O-specific side chain responsible for its immunological properties and a lipid A region thought to be responsible for the pyrogenic characteristics of the molecule (Work, 1971). The effects of endotoxins on the organism include protection against irradiation, enhancement of nonspecific immunological resistance, reduction in serum iron levels, lowered blood pressure and fever (Kluger, 1979). Both live and dead gram negative bacteria are capable of causing fever. Repeated injection of endotoxin will also result in the animal developing a tolerance to the endotoxin.

b) Endogenous pyrogen

One of the properties of exogenous pyrogens in the body is the activation of the reticulo-endothelial system. Phagocytic cells destroy and engulf the exogenous pyrogen molecule and in the process release another distinctly different pyrogenic material into the circulation, endogenous pyrogen (EP). Cells capable of producing EP include peritoneal exudate granulocytes and macrophages, peripheral blood neutrophils, blood monocytes, eosinophils, alveolar macrophages

and fixed tissue macrophages such as splenic sinusoidal cells and hepatic Kupffer cells (Dinarello, 1979).

The endogenous pyrogen produced from phagocytic cells under the influence of endotoxin is a protein which has proven difficult to purify. Recently however, a molecule or possible family of molecules called interleukin I has been described which derives from phagocytic cells in response to infection and appears to be identical to EP. At present there is no known sequence analysis for EP or interleukin I (Reviewed in Dinarello, 1984). It has also been shown that EP produced by different cell types in the same animal are not all the same and cause different patterns of fever. However, EP from one species will still cause fever in another (Dinarello, 1979).

c) Site and mechanism of action of EP

Evidence has accumulated which indicates that the POAH region is the target of the EP molecule. Although it was previously believed that EP is incapable of crossing the blood brain barrier, it has recently been shown that EP may gain direct access to the POAH region through the organum vasculosum of the lamina terminalis. Blatteis et al (1983) reported that lesions of this circumventricular organ resulted in the prevention of EP induced fevers. They suggested that this was due to inability of EP to cross the blood brain barrier (BBB) and not to the impairment of the febrile mechanism since EP microinjected directly into the medial preoptic area of lesioned animals resulted in typical febrile responses. Other evidence also suggests that EP exerts its pyrogenic action directly on neurons in the hypothalamus. Electrophysiological studies reveal that both peripherally administered EP (Eisenman, 1969; Cabanac et al, 1968) and

EP microinjected into the POAH and septal regions (Wit and Wang, 1968b; Schoener and Wang, 1975) causes depression of activity of warm sensitive neurons and enhancement of firing of cold sensitive neurons in these areas. Boulant and Scott (1983) recently report that the major response of thermosensitive neurons in the hypothalamic slice to bath application of EP was an increased firing in warm sensitive, decreased firing in cold sensitive and no response in thermally insensitive units. These responses are appropriate for the decrease in heat loss and increase in heat production observed during the rising phase of fever.

Although these studies implicate the POAH as the central site of action of EP, Veale and Cooper (1975) have shown that intravenously administered EP is capable of causing fevers in animals with POAH lesions. Dinarello et al (1978) also report that human purified radiolabelled EP injected intravenously in rabbits was not detectable in the POAH 30 minutes later even though a fever developed. This suggests there may be other, as yet unidentified, pyrogen sensitive brain regions.

It has also been suggested that EP may not cross the BBB, but act through a mediator substance to indirectly cause increases in body temperature. A large body of evidence has been put forward to implicate prostaglandins of the E series (PGE) as the intermediary in EP induced fevers. First, PGE2-like activity is found in elevated levels in cerebral spinal fluid during BP or EP induced fevers (Feldberg and Gupta, 1973; Philipp-Dormston and Siegert, 1974). In addition, the observation that microinjection of PGE2 in the ventricles and POAH specifically cause an increase in body temperature

(Milton and Wendlant, 1971; Feldberg and Saxena, 1971; Williams et al, 1977) is further supported by electrophysiological data demonstrating that ventricular infusion and iontophoresis of prostaglandins in the POAH facilitates cold sensitive and inhibits warm sensitive neurons (Ford, 1974; Gordon and Heath, 1979). This effect is consistent with the data from studies of the effect of EP on thermosensitive neurons (Eisenman, 1969; Cabanac et al, 1968; Wit and Wang, 1968b; Schoener and Wang, 1975). As well, antipyretics such as salicylates and allied drugs, inhibit prostaglandin synthesis in vivo (Vane, 1971) and, when administered during fever, concommitantly attenuate the fever and reduce the levels of PGE2-like activity in cerebral spinal fluid (Feldberg and Gupta, 1973). The pyrogen induced inhibition of warm sensitive POAH neurons was further shown to be reversibly blocked by local application of salicylate (Hori et al, 1984; Wit and Wang, 1968b).

The evidence of a prostaglandin mediated EP fever however has also been questioned. Electrophysiological findings have not been consistent with the work of Ford (1974) and Gordon and Heath (1979) and report that only 10% of thermosensitive or thermoresponsive POAH neurons respond to microinjection or iontophoretic application of prostaglandins and not in a consistent manner (Jell and Sweatman, 1977; Stitt and Hardy, 1975). Cranston et al (1975) reasoned that if prostaglandin synthesis formed an essential link in the action of pyrogens in the brain, then fever and brain prostaglandin synthesis must always be associated. They showed that the elevated cerebral spinal fluid levels of PGE2 commonly observed during fever could be completely prevented by infusion of salicylate in doses which did not

affect the development of fever. Further, they were also able to show that injection of two separate PGE2 antagonists reduced PGE2 fevers but had no effect on EP induced fevers (Cranston et al, 1976). These authors therefore concluded that any brain prostaglandin synthesis observed during an EP fever does not play an essential role in the genesis of fever. Coceani et al (1983) have further contributed to this controversy by showing that the methods of measuring PGE2 in earlier experiments have been inaccurate. They propose that another arachidonic acid metabolite, thromboxane A2, may also be important to the development of a pyrogen fever. Radioimmunoassay of cerebrospinal fluid samples during an EP induced fever showed increased thromboxane A2 levels and PGE2 levels which corresponded to the rise in body temperature. Although far from conclusive, this work demonstrates that other arachidonic acid metabolites also subject to inhibition of synthesis by aspirin like drugs (cyclo-oxygenase inhibitors) may play a role in mediating EP fevers.

A separate line of research suggests that the mechanism by which EP causes fever is through some process involving protein synthesis. It has been reported that a protein synthesis inhibitor, cycloheximide, can reduce fever caused by centrally administered EP or arachidonic acid (Cranston et al, 1983; Hellon et al, 1984). The precise mechanism by which cycloheximide interferes with the action of EP on its target tissue in the POAH has not yet been studied.

iii) Antipyretics

a) Site of action

"Substances which reduce the temperature in febrile and similar states but not in normal conditions, unless the dosage be excessive,

are termed antipyretics." This definition of antipyretics was proposed by Barbour (1921) and is still accepted as accurate today. Antipyretic drugs may be steroidal or non-steroidal in nature. The majority of study on antipyretics has been done using non-steroidal antipyretic drugs and as yet no clear mode of action has been established to explain how these antipyretic substances lowers the febrile temperature.

Hypothetically, antipyretics may interfere with the development of fever at any point along the febrile pathway. This was the premise for a number of investigations into antipyretic action. Consequently it has been shown that antipyretics do not interfere with the production or release of EP (Rosendoreff et al, 1979; Van Miert et al, 1971; Cranston et al, 1970; Lin and Chai, 1972), nor do they inactivate the EP molecule (Hoo et al, 1972; Lin and Chai, 1972). Although it has not been clearly established whether EP enters the brain itself or acts through an intermediary, Cooper et al (1968) have reported that intravenous salicylate attenuated EP fever while intracerebral ventricular salicylate did not. These authors therefore proposed that antipyretics may act by limiting the passage of EP into the pyrogen sensitive area in the hypothalamus. Subsequent experiments did not confirm these observations but instead, showed that the amount of intracerebral ventricular salicylate administered which was required to reduce fever was far less than the amount of salicylate required intravenously (Cranston et al, 1971; Lin and Chai, 1972; Clark and Alderdice, 1972). Salicylates were therefore proposed to exert their antipyretic activity at the same site as pyrogens exert their pyretic activity in the hypothalamus. The identification of the

POAH as the site of exogenous antipyretic action was made by Cranston et al (1970) who showed that microinjection of antipyretics were most potent in the anterior hypothalamus and by Lin and Chai (1972) who reported that antipyretics microinjected into the POAH dramatically reduce the dosage of antipyretics required intravenously to attenuate fever. Direct evidence also indicates that salicylates microinjected into the POAH inhibit the effects of EP microinjected into the same area (Schoener and Wang, 1975).

b) Mechanism of action

Studies on the mode by which antipyretics reverse the pyrogen effects in the POAH during fever are limited by the inability to determine the mechanism by which pyrogens act to cause the increase in body temperature initially.

It is still not conclusive that fever is mediated by prostaglandins, however, strong evidence exists for antipyretics acting to inhibit EP stimulated prostaglandin synthesis or release in the POAH. Antipyretics have been shown to inhibit prostaglandin synthetase (cf Rosendorff and Woolf, 1979) and Ziel and Krupp (1975) have found a significant correlation between the antipyretic activity and prostaglandin synthetase activity in the rat. Antipyretics do not antagonize the effects of prostaglandins once synthesized (Woolf et al, 1975).

Antipyretics may also act by antagonizing any direct effects of EP on POAH neurons. It is well documented that pyrogens can alter the firing of central thermosensitive neurons (see section ii(c)). Many authors also report that intravenously administered salicylates act as antipyretics by reversing the effects of pyrogen on thermosensitive

cells in the POAH (Wit and Wang, 1968b; Cabanac et al, 1968; Eisenman, 1969). These data are supported by microinjection (Schoener and Wang, 1975) and in vitro studies (Hori et al, 1984) in which salicylates were shown to reverse the EP induced changes in thermosensitive neuronal firing. In most cases salicylates were also shown to be ineffective without EP pretreatment (Wit and Wang, 1968; Schoener and Wang, 1975; Hori et al, 1984).

However, iontophoretic application of salicylates has also been shown to cause changes in the firing of POAH neurons in the absence of pyrogens (Beckman and Roskowska-Ruttiman, 1974; Jell and Sweatman, 1976). When the thermosensitivity of hypothalamic neurons was studied, it was found that warm sensitive cells were predominantly excited by salicylate application (Beckman and Rozkowska-Ruttiman, 1974). These authors suggested a mechanism whereby antipyretics act on warm sensitive neurons to stimulate heat loss processes independent of EP activity. This mechanism is also supported by observations that some antipyretics lower the body temperature in normothermic animals (Bruns et al, 1950; Milton, 1973; Feldberg and Saxena, 1975). However, since other investigators have not been able to repeat these results (Pittman et al, 1976; Rosendorff and Cranston, 1968), it has been argued that the hypothermic effects of antipyretics observed are unique cases and may be due to nonspecific activation of other systems (Rosendorff and Wolff, 1979).

The concept that EP and antipyretics act on the same neuron has been further examined pharmacologically. Changes in log-dose response curves of EP with antipyretic pretreatment suggests that these two substances may act as competitive antagonists (Clark and Caldwell,

1972; Lin and Chai, 1972). However, structure-activity relationships do not favor the hypothesis that substances such as salicylate, indomethacin and anthranilic acid derivatives compete with EP for the same receptor sites. Therefore, although the consensus in the literature appears to be that antipyretics act primarily in the POAH to antagonize the effects of EP on thermosensitive units, the precise manner in which pyrogens alter thermosensitive neurons to effect an increase in body temperature and in which antipyretics reverse this action remains to be determined.

iv) Endogenous Antipyresis

It has long been recognized that fevers rarely exceed 4 C above normal body temperature (Banet, 1979; Bligh, 1973). This suggests that there must be some form of negative feedback loop which controls the magnitude of the febrile response. It has also been observed that while the thermoregulatory mechanism of newborn, near term pregnant and postpartuitant animals have all been shown to be intact, these animals do not express an increased body temperature in response to pyrogen challenges (Kasting, 1980; Pittman, 1976). It was suggested that this natural suppression of fever indicated an endogenous antipyretic component to central nervous system thermoregulatory processes not previously considered (Kasting, 1980).

Arginine vasopressin (AVP) has recently been proposed as a candidate responsible for mediating endogenous antipyresis (Cooper et al, 1979). The selection of this peptide followed from observations that plasma AVP levels in near term pregnant ewes and fetuses were elevated (Alexander et al, 1974) and that local heating of the POAH and ventrolateral septal areas in nonpregnant dogs also resulted in

elevated AVP levels (Szczepanska-Sadowska, 1974). This suggested that elevated plasma levels of AVP may be related to a possible humoral agent which could suppress fever centrally (Cooper et al, 1979).

Arginine vasopressin was proposed as the substance mediating the observed antipyresis when it was shown that AVP perfused in the septal region of nonpregnant febrile sheep resulted in a dose dependent suppression of fever but not of normal body temperature (Kasting, 1980). Evidence for central release of AVP during antipyresis was provided by further studies in which radioimmuno-assays for AVP of push pull perfusion ventricular and ventroseptal samples showed a negative correlation with body temperature in febrile animals. This demonstrated that the decrease in body temperature during the falling phase of fever was associated with increased release of immunoreactive AVP in the septal area (Cooper et al, 1979).

Anatomical evidence for an increased release of vasopressinergic neurons during antipyresis was provided by Zeisberger et al (1981) and Merker et al (1980) who also observed suppression of fever in near term pregnant and post parturitant guinea pigs. Immunohistochemical studies were used to demonstrate an increase in immunoreactive AVP centrally in the cell bodies of the paraventricular hypothalamic nucleus (PVN) and in fibers of the lateral septum and amygdala in pregnant guinea pigs which did not demonstrate a fever when given exogenous pyrogen. These sites demonstrating increased immunoreactive AVP activity corresponded to the site of action of AVP antipyresis and to other anatomical studies showing that the putative source of AVP projecting to the septal region may be the PVN (Buijs et al, 1978).

Glyn and Lipton (1981) and Bernadini (1983) have challenged these observations and report that microinjection of AVP into the lateral septum results in an increase in body temperature in rabbits. These authors suggested that the antipyretic action of AVP was specific to the sheep and not other species. However, it has now been demonstrated that AVP will act to reduce fevers caused by EP or prostaglandins in the sheep (Kasting, 1980), cat (Ruwe et al, 1985b), rat (Ruwe et al, 1985a), and rabbit (Naylor et al, 1985a). This discrepancy may be due to the site of application of AVP used by Lipton and his colleagues (1980, 1983). It has now been confirmed that the site of action of AVP is the ventral septal area (VSA) precisely (Naylor et al, 1985b). Microinjection of AVP into the more dorsal septal area or ventricles could either neglect to activate appropriate neurons involved in antipyresis or would expose numerous other brain regions to AVP activity and cause a nonspecific effect. Studies by Lee and Lomax (1982) supported the required specificity of the site of AVP's antipyretic activity when they reported that AVP administered intracerbroventricularly caused a reduction in the body temperature of afebrile gerbils while others (Kasting, 1980, Naylor et al, 1985b) showed that AVP in the VSA specifically had no effect on the normal body temperature of a number of animals at room temperature.

The mechanism by which vasopressin may cause antipyresis has only recently been studied. Banet and Wieland (1985) have provided evidence for vasopressin acting to inhibit thermoregulatory heat production but not reduce the normal set point temperature. This work did not investigate the possibility that vasopressin may also prevent

the febrile rise in set temperature or alter other parameters involved in the febrile response. Therefore, although the site of action has been identified, the mechanism of AVP antipyresis has yet to be elucidated.

C. AVP as a Central Neurotransmitter

Arginine vasopressin is a nonapeptide which was originally recognized for its peripheral antidiuretic and pressor hormonal effects. This hormone was initally identified centrally in the neurohypophysis by Verney (1947) and later in the PVN and supraoptic nucleus of the hypothalamus (Olivercrona, 1957; Zimmerman et al, 1974). Recognized as components of the hypothalamo-neuropophyseal system, neurons from the PVN and supraoptic nucleus (SON) project to the neural lobe of the pituitary where, upon appropriate stimulation, AVP is released from the axon terminal into the circulating blood. The peripheral endocrine actions of AVP include antidiuresis, vasoconstriction (Saameli, 1968), increased glycogenolysis and neoglucogenesis (Hemms and Whitton, 1973), platelet aggregation (Haslam and Rosson, 1972) and mitogenic effect on several cell types (Hunt et al, 1977; Miler et al, 1977; Rozengurt et al, 1979).

Advances in anatomical techniques have since permitted for the identification of vasopressin in numerous brain regions, suggesting that it may exert central neuromodulatory as well as peripheral hormonal actions (George and Jacobowitz, 1975; Buijs, 1978; Sofroniew and Weindl, 1978). Vasopressin has now been shown to be active in a number of centrally mediated events including antipyresis (Cooper, 1979), behavioral (DeWied, 1976), convulsive activity (Kasting et al,

1980) and regulated cardiovascular function (Pittman et al, 1982), and fulfills many of the criteria required for its classification as a neurotransmitter in the brain.

i) Anatomy

a) Cell bodies

The primary sources of extrahypothalamic AVP in the brain were originally thought to be the PVN and suprachiasmatic nucleus (SCN). Although immunocytochemical techniques have confirmed cell bodies containing immunoreactive AVP in these defined nuclei, more recent studies indicate immunoreactive AVP in perikarya of the supraoptic nucleus, bed nucleus of the stria terminalis (BST), medial amygdala, ventral lateral septum and locus coeruleus (Buijs et al, 1978, Buijs and Swaab, 1979; Van Leeuwen and Caffe, 1983; DeVries and Buijs, 1983; Sofroniew and Wiendl, 1978). All of these regions also stain for neurophysin which indicates the synthesis of AVP in the perikarya of these nuclei. In addition, accessory magnocellular AVP neurons have also been found to be distributed throughout the hypothalamus in undefined regions of the anterior hypothalamus between the PVN and supraoptic nucleus and the posterior hypothalamus (Sofroniew, 1985).

b) Fibres

The most clearly defined projection of vasopressin fibres is to the capillaries of the posterior pituitary for release into the vascular circulation. The origin of these fibres is believed to be the PVN, supraoptic nucleus and hypothalamic accessory magnocellular groups exclusively (Sofroniew, 1985). A prominent projection arising from the PVN to the portal capillaries of the median eminence has also been described. As well, AVP containing fibre pathways which appear

unrelated to neuroendocrine functions have been identified. The highest brain concentration of AVP containing fibres is found in the subcortical structures, particularly the forebrain limbic structures such as the lateral septum, lateral habenular nucleus and amygdala (Sofroniew, 1983). AVP immunoreactive fibres have also been detected in smaller amounts in numerous midbrain and hindbrain areas and in the spinal cord.

c) Pathways

The precise origins and projections of AVP containing neurons has not been elucidated in most brain and spinal cord areas known to contain AVP. However, using immunocytochemistry along with retrograde transport, lesion and electrophysiological studies, it has been convincingly demonstrated that PVN vasopressinergic neurons project to the nucleus tractus-solitarius and dorsal vagal nucleus of the brainstem, where AVP has been implicated in cardiovascular function (Sawchenko and Swanson, 1982; Sofroniew and Schell, 1981; Hosoya and Matsushita, 1979; DeVries and Buijs, 1983; Lawrence and Pittman, 1984; Pittman and Franklin, 1985). The PVN also appears to be the source of AVP in the spinal cord (Pittman et al, 1984). More recently the vasopressin projections to the septal area where AVP mediated antipyresis has been shown has also been investigated.

The PVN has been shown to project to numerous brain regions and was believed to be the major source of extrahypothalamic AVP in the brain. Pittman et al (1981) provided evidence for a PVN-septal projection when they described antidromic responses from stimulation of the lateral septum while recording extracellularly from the PVN. However, lesion studies indicate that the AVP content of this pathway

may be in question (DeVries and Buijs, 1983). Immunohistochemistry has provided evidence for a suprachiasmatic nucleus-lateral septal AVP projection (Sofroniew and Wiendl, 1978) but lesions of this area has not been shown to alter the AVP content of the septal region (Hoorneman and Buijs, 1982). The BST has also been investigated as a major source of AVP in the septum. Both lesion studies and isolation of this area with knife cuts suggests that the BST may contribute substantially to septal vasopressin innervation (DeVries and Buijs, 1983; VanLeeuwen and Caffe, 1983). This projection is further supported by studies in which retrograde labelling of the lateral septum from the BST has been shown (DeVries and Buijs, 1983). No electrophysiological work as yet has confirmed this projection.

ii) Synthesis

The synthesis of vasopressin has been extensively studied in the hypothalamo-neurohypophysial system. It is now understood that the peptide molecule AVP derives from a precursor molecule synthesized in the magnocellular cells of the PVN and supraoptic hypothalamic nuclei. Recently, recombinant genetic techniques have been used to characterize the structure of the gene which encodes for the precursor of AVP (Schmale and Richter, 1984). The initial expression of the gene responsible for AVP is a pre-pro-hormone synthesized on the ribosomes. Translational modifications result in a prohormone consisting of the AVP peptide sequence, a neurophysin carrier protein and a glycoprotein. The prohormone is packaged in a neurosecretory granule where it is further processed into the three separate components during axonal transport to the terminal. AVP and its neurophysin are then either stored in the terminal or released from

the granules via a Ca++ dependent exocytotic mechanism in the posterior pituitary.

The recent discovery of extra-hypothalamic AVP containing cell bodies in the central nervous system indicates that AVP biosynthesis also occurs outside the hypothalamus. Although extensive biochemical analysis of the AVP found in extrahypothalamic areas has yet to be done, evidence suggests that it may be synthesized in a manner similar to that found in the hypothalamus (Pickering et al, 1983).

iii) Release

Another neurotransmitter criteria is the demonstration of release of the transmitter substance from nerve terminals upon depolarizing stimulation. Immunocytochemistry combined with electron microscopic visualization has identified immunoreactive AVP containing terminals making synaptic connections with the dendrites of neurons in the lateral system, lateral habenular nucleus and amygdala (Buijs and Swaab, 1979). These synapses appear to be fundamentally identical to the classical transmitter-containing synapses in the brain. With veratridine or a high K+ solution for depolarizing stimuli, Ca++ dependent AVP release was demonstrated in regions showing these synaptic connections (Buijs and Van Heerikhuize, 1982; Disturnal et al, 1983). Immunoreactive AVP release in response to electrical stimulation of the PVN has also been demonstrated in the spinal cord (Pittman et al, 1984).

Vasopressin release under the physiological condition of fever has also been demonstrated. Cooper et al (1979) showed that the amount of immunoreactive AVP present in push pull perfusion samples of the ventral septal area dramatically increased during fever. In vivo

studies by Kasting and Martin (1982) have also shown changes in brain concentrations of AVP-like immunoreactivity resulting from endotoxin induced fever in the rat. These authors reported decreased AVP concentrations in several brain regions including the septal area which were interpreted to indicate endotoxin stimulated release of AVP in these regions.

iv) Receptors

Vasopressin receptors have been well characterized in isolated cells or membrane fractions derived from three AVP-responsive tissues; kidney, liver and blood vessels. In each case it has been confirmed that binding sites studied are the receptors involved in the biological effects of vasopressin on these tissues (Jard, 1983). In 1979 Michell et al proposed that at least two types of AVP isoreceptors existed which could be classified according to the nature of the effectors to which these receptors were coupled. Renal receptor activation involved high affinity binding of AVP to a cyclic AMP dependent (V2) receptor while extrarenal receptors bound with a ten times lower affinity to a cyclic AMP independent, Ca++ dependent (V1) receptor. In addition, the different potencies of synthetic AVP agonists and antagonists that exert preferentially pressor (V1 receptor) or antidiuretic (V2 receptor) actions have also been described (Sawyer et al, 1981a, 1981b).

These characteristics of AVP receptors have served as the basis of characterization for the AVP receptors recently identified in the central nervous system. The use of autoradiography to identify [3H]AVP-labelled binding sites in the brain has revealed intense labelling in the PVN and supraoptic nucleus of the hypothalamus

(Yamamura et al, 1983) and extrahypothalamically in the septum, hippocampus, olfactory nucleus, amygdaloid nucleus and nucleus tractus solitarius of the brainstem (Van Leeuwen and Walters, 1983; Beigon, 1984; Baskin et al, 1983; Dorsa et al, 1983, 1984). This labelling corresponds well with the location of AVP content and actions in the central nervous system and is particularly interesting in the septal area where AVP containing synapses have also been demonstrated (Buijs and Swaab, 1979).

The mechanism by which central receptors are activated has not yet been determined. Courtney and Raskind (1983) have shown that cyclic AMP is not stimulated in several brain regions by central administration of AVP. However, in the caudate nucleus, AVP greatly enhanced the activation of adenylate cyclase by dopamine. This indirect activation of cyclic AMP by AVP may explain the increased levels of cyclic AMP in the brain following peripheral AVP administration as observed by Schneider et al (1982). The only direct evidence to date which suggests that central receptors are similar to the cyclic AMP dependent V2 receptor comes from the work of Abe et al (1982) who were able to demonstrate that AVP and dibutyrl cyclic AMP caused similar neuronal membrane effects and a cyclic AMP stimulator (adenosine) also produced electrophysiological effects resembling those of vasopressin in the supraoptic nucleus.

Recently, peripherally active AVP selective agonists and antagonists have been used to study the characteristics of central AVP receptors. Results indicate that the renal (V2) agonists had no effects centrally on cardiovascular (Riphagen and Pittman, 1985) or convulsive behavior induced by AVP (Burnard et al, 1985). This

coincides with the inability of investigators to show AVP stimulated increased cyclic AMP levels in various brain regions and supports the proposal that central receptors are not similar to the renal (V2) receptor.

Pressor (V1 receptor) antagonists have, on the other hand, been shown to be capable of blocking the effects of AVP on cell firing in the hippocampus as well as the behavioral convulsive and cardiovascular effects of centrally administered AVP (deWied et al, 1984; Pittman and Franklin, 1985; Burnard et al, 1985; Tiberiis et al, 1983). These results suggest that central AVP receptors may resemble the pressor (V1) receptor. However, it is also very likely that several different subtypes of AVP receptors exist in the CNS which have yet to be defined since a fragment of AVP which is a potent activator of behavioral responses is ineffective in eliciting antipyretic actions in the rat (Kovacs and DeWied, 1983). Further an AVP derivative, [pGlu4,Cyt6]AVP4-8, which has central behavioral effects but no peripheral pressor actions can be blocked by a pressor antagonist (deWied, 1984).

v) Inactivation of AVP

Neurotransmitter criteria also requires a mechanism for inactivation. It was previously assumed that neuropeptide molecules are inactivated by proteolytic enzymes commonly found in abundance in the brain. However, the recent observation that both the intact nonapeptide AVP and fragments of this molecule exert centrally mediated behavioral responses (DeWied, 1976; Kovacs and DeWied, 1983) suggests that proteolytic processes specific to AVP may exist in the brain. Burbach and Lebouille (1983) have shown that synaptic membrane

associated proteolytic processes cleaves both the AVP and related oxytocin molecule. Aminopeptidases not previously characterized in the brain have been identified as being responsible for the proteolytic mechanisms which produce neuroactive AVP fragments. Further activity by these aminopeptidases also produces AVP fragments which have no known central actions. This process may be responsible for the formation of active AVP fragments and the later inactivation of these molecules.

vi) AVP Actions on Central Neurons

The actions of vasopressin in the central nervous system have been studied in both mammalian and invertebrate preparations with variable and often directly contrasting results. The confusion surrounding the actions of AVP observed is further complicated by the fact that although evidence for central AVP receptors exists, the classification of these receptors has not yet been clarified.

The hippocampus has been shown to be innervated by AVP containing fibres and the actions of AVP in the slice preparation of this structure has been extensively studied. Muhlethaler et al (1982) originally reported that AVP caused an increase in the firing rate of pyramidal CA1 hippocampal neurons. A later report (Muhlethaler et al, 1983) revealed that AVP had excitatory effects on nonpyramidal hippocampal cells but that the receptor mediating the action of AVP preferentially recognized a related neurohypophysial peptide, oxytocin. Tiberiis et al (1983) observed similar excitatory effects of both oxytocin and AVP on identified CA1 hippocampal neurons. Since two different AVP antagonists were shown to block the response to both AVP and oxytocin, they suggested that the observed effects of AVP and

oxytocin were mediated by the same receptor. However, since these authors did not measure cyclic AMP or Ca++ activation, the receptor population involved in these responses remains to be determined.

Muhlethaler et al (1984) more recently reported that AVP caused a direct excitation of nonpyramidal inhibitory interneurons and provided intracellular evidence for indirect inhibitory effects of AVP on CA1 pyramidal cells. Intracellular recordings of CA1 pyramidal cells by Mizuno et al (1984) also indicate that AVP effects on these neurons are transsynaptic although their results show a resultant excitatory action of AVP. As yet there is no explanation for these opposing reports.

Perhaps the most extensive intracellular study on vasopressin actions was by Abe et al (1982) using the guinea supraoptic nucleus slice preparation. These authors showed that AVP had a direct effect of inhibiting the spontaneous activity of supraoptic neurons. As also shown by Mizuno et al (1984), their work revealed that AVP depolarized the neuronal membrane without altering input resistance. The observed AVP response was also unaffected by changes in external K+ or by depletion of external Ca++. Alterations in external Na+ however significantly modified the effects of AVP. They further indicated that a V2-like receptor mediated these effects by showing that dibutyrl cyclic AMP depolarized the membrane in a manner similar to AVP, phosphodiesterase inhibitors potentiated AVP's effects and increased levels of cyclic AMP could be seen in hypothalamic slices incubated with AVP containing medium. Based on their results, these authors proposed a mechanism whereby a specific membrane protein phosphorylated by cyclic AMP may be associated with a carrier

mediated, active entry of sodium into the intracellular compartment. Further work is needed to substantiate this attractive hypothesis.

The possibility that AVP's actions may involve other neurotransmitters has also been studied. AVP has been shown to enhance catecholamine disappearance in a number of brain regions including the septum, amygdala and nucleus tractus solitarius (Tanaka et al, 1977). A dose dependent increase in dopamine utilization was also found in the amygdala following local microinjection of AVP (VanHeuven-Nolson et al, 1984). It has also been reported that AVP can potentiate noradrenaline-stimulated cyclic AMP accumulation in the mouse hippocampus (Church, 1981). Since the hippocampus shows rich noradrenaline as well as AVP innervation, these observations suggest a possible interaction between noradrenaline and AVP at synaptic sites on hippocampal neurons.

Iontophoretic studies by Olpe and Baltier (1981) indicate that AVP causes an increase in the firing rate of noradrenergic neurons of the locus coerulus. Fewer cells responded when AVP was applied to non noradrenergic neurons. In the septum, Joels and Urban (1982) reported a slight excitation of AVP on neurons of this area but a predominant effect of enhancement of glutamate excitation. These results were further supported by field potential studies (Joels and Urban, 1984). Marchand and Hagino (1982) also iontophoretically applied VP in the septal area and noted a reversible suppression in the amplitude of field potentials and, in single unit studies, an inhibition of fimbria-fornix excitation (believed to be glutamate mediated).

It is apparent that the effects of AVP on central neurons have not been clearly defined. It is conceivable that AVP may exert

different actions within the CNS but the understanding of the different mechanisms by which AVP may act as a neurotransmitter or neuromodulator is presently hindered by the inability to define the receptors mediating central AVP activity.

D. Rationale for Research

This research was undertaken in an attempt to understand the neuronal mechanisms at the single unit level which underlie the antipyretic activity of arginine vasopressin in the brain. That the complex phenomenon of endogenous antipyresis occurs specifically in the ventral septal area signifies this region as a major component of central thermoregulatory systems and introduces a new concept in the basic physiological mechanisms behind central nervous system control of thermoregulatory and febrile processes. The ventral septal region had not previously been recognized as independently responsible for any significant thermoregulatory action. Further evidence implicating this region in the thermoregulatory pathways is now needed. In addition, the physiological response of antipyresis mediated by endogenous arginine vasopressin is dependent on the ability to prove that this peptide acts as a neurotransmitter or neuromodulator in the ventral septal area. Preliminary studies suggest a role for AVP as a neurotransmitter in the central nervous system however, conflicting reports surrounding the physiological responses resulting from AVP activity in the septal area indicate that the status of AVP as a putative neurotransmitter has not been firmly established and also requires further study.

It is hypothesized that AVP acts as a neurotransmitter or neuromodulator in the ventral septal area of the brain which relates to its observed actions as an antipyretic. Experiments were therefore designed to achieve the following objectives:

1. To electrophysiologically identify the potential sources of endogenous AVP in the ventral septal area

2. To determine if neurons in the ventral septal area are thermoresponsive and therefore involved in thermoregulatory pathways.

3. To determine if thermoresponsive neurons receive afferent projections from the potential sources of AVP in the ventral septal area.

4. To determine the single unit response of ventral septal area thermoresponsive and nonthermoresponsive neurons to AVP application and relate this with innervation from the potential sources of AVP.

The rat was used as the animal model for this research because:

1. Rats are well characterized with respect to their brain stereotaxis.

2. AVP antipyresis has previously been demonstrated in the rat.

II. ELECTROPHYSIOLOGICAL EVIDENCE FOR ARGININE VASOPRESSIN PROJECTIONS TO THE VENTRAL SEPTAL AREA OF THE BRAIN

A. Introduction

The observation that AVP acts in the VSA to cause antipyresis during fever suggests that this peptide is a neurotransmitter in this area of the brain. Yet an endogenous source of AVP to its site of action in the VSA remains to be identified. Recently, anatomical studies have identified a number of brain regions containing AVP-immunoreactive cell bodies (Sofroniew, 1985). However, the central projections and termination of these AVP containing cells has not been established. With respect to the septal area, lesion studies suggest that the BST is the source of AVP innervation of the lateral septum (DeVries and Buijs, 1983; Hoorneman and Buijs, 1982). However, the innervation of the more ventral septal area and the diagonal band of Broca (DBB) where the antipyretic action of AVP has been localized has received less study. Axonal transport studies indicate projections to these areas from the PVN, BST and SCN (Conrad and Pfaff, 1976a; DeVries and Buijs, 1983; Swanson and Cowan, 1975) but the potential involvement of AVP in these projections is not known.

This study was carried out to examine in more detail the potential AVP projections to the VSA and to identify the possible inhibitory or excitatory nature of these afferents. As well, preliminary information as to the interactions among putative AVP afferents and other known afferents to the VSA were studied.

B. Experimental Procedures

Experiments were conducted on male Sprague Dawley rats (250-300 grams) anaesthetized with urethane (1.6 g/kg). Body temperature was maintained constant at 37°C throughout the experiment by means of a feedback-controlled heating blanket. Bipolar axial stimulating electrodes (nichrome wire, 200 μm OD, tip separation < 50 μm) were implanted stereotaxically in the PVN, BST, fornix and amygdala. Electrodes were connected to isolated stimulation units which delivered sequential monophasic current pulses (0.2 ms. duration, 0.1-0.4 mA a 1Hz).

A transpharyngeal approach was then used to expose the ventral surface of the brain and a concentric bipolar stimulating electrode was positioned by micromanipulation in the SCN. Extracellular recordings were obtained through a pontamine sky blue/Na+ acetate dye filled glass micropipette (5-10 Mohms impedence) passed from the ventral surface through the DBB, VSA and into the lateral septum. The electrode was positioned with reference to the midline, the posterior border of the optic chiasm and the ventral surface of the brain.

Following standard amplification and filtering, and, on occasion, photography of the recorded potential on the oscilloscope screen, a variable voltage gate was used to select action potentials for spike train analysis (post stimulus histograms) on a PDP 11/23 computer. Orthodromic responses were classified as excitatory when the initial response to the stimulus was an evoked potential of variable latency and inhibitory when a spontaneously active neuron displayed an abrupt decrease in excitability after the stimulus. Antidromic responses were characterized using the following criteria: 1) A constant latency

action potential which follows threshold stimulation intensity; 2) Following at high frequency (200 Hz) stimulation and 3) In the case of spontaneously active cells, collision cancellation between a spontaneous and an antidromically evoked action potential.

At the termination of an electrode penetration, the tracts and all sites of interest were marked by iontophoresis of pontamine sky blue dye. Histological verification was made of all stimulating and recording sites.

C. Results

Ninety eight neurons throughout the DBB, VSA and lateral septum were tested for their response to PVN stimulation, and 64 of these displayed evoked responses (Fig 1). The PVN accounted for the largest afferent input of the potential sources of AVP studied with 71% of evoked potentials being orthodromic in nature. Inhibitory or excitatory orthodromic responses were equally represented in the population of evoked responses. In general, orthodromic responses occurred after a latency of 5-20 msecs. When the locations of neurons receiving orthodromic input from the PVN were plotted in coronal diagrams of the ventral septal area, it became apparent that a large population of cells was located in the VSA, whereas, the lateral septum population of such cells was relatively less populous. In contrast, the 21 cells which displayed antidromic potentials following PVN stimulation were largely localized to the lateral septum.

Forty-four VSA and lateral septal neurons displayed evoked potentials following stimulation of the BST (Fig 2). Orthodromic responses (equally excitatory and inhibitory) represented 85% of the total number of evoked responses. Twelve neurons which displayed

FIG. 1

A. Coronal sections of rat brain 0.2 and 0.7 mm rostral to bregma on which are plotted cells which responded to PVN stimulation with antidromic invasion (*) orthodromic excitation (o) or orthodromic inhibition (●). (LS, lateral septum; AC, anterior commissure; DBB, diagonal band of Broca).

B. Oscilloscope trace of 10 superimposed sweeps demonstrating orthodromic excitation of a unit in the VSA following electrical stimulation of the PVN at 1.0 Hz. Arrow denotes stimulus artifact.

C. Oscilloscope trace of 5 superimposed sweeps demonstrating antidromic delivery of a paired 200 Hz stimuli (arrows) to the PVN.

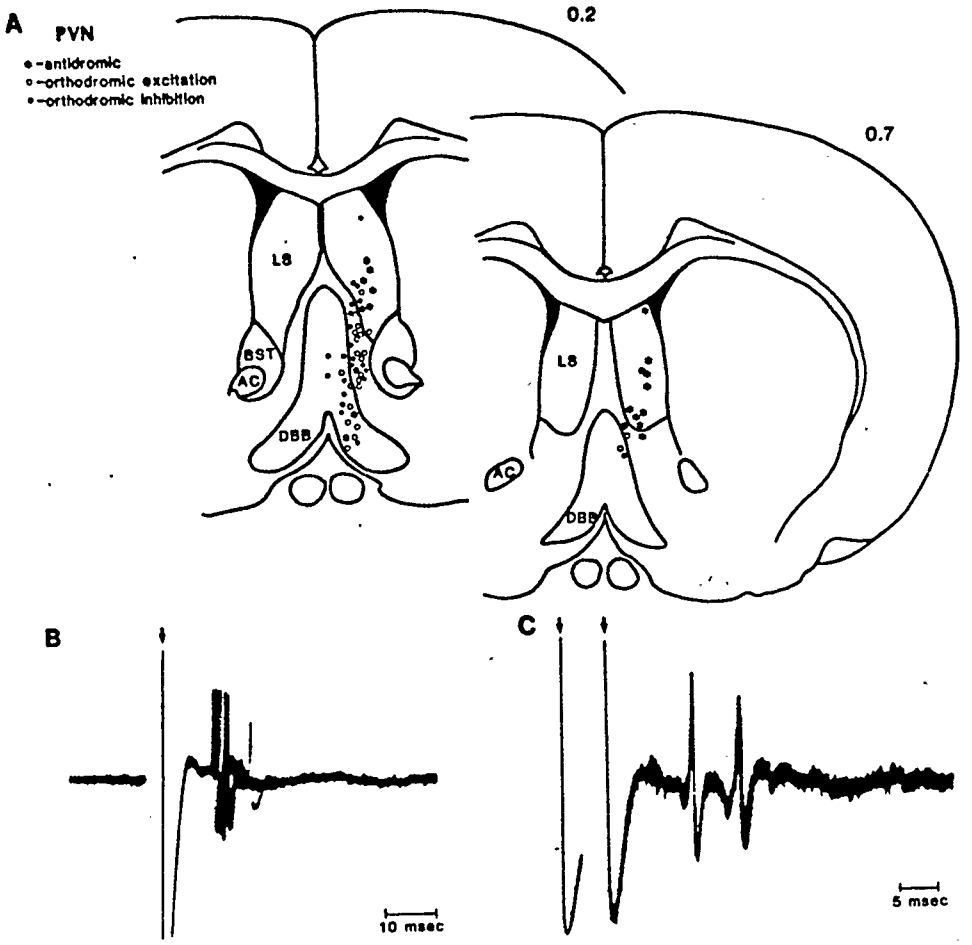
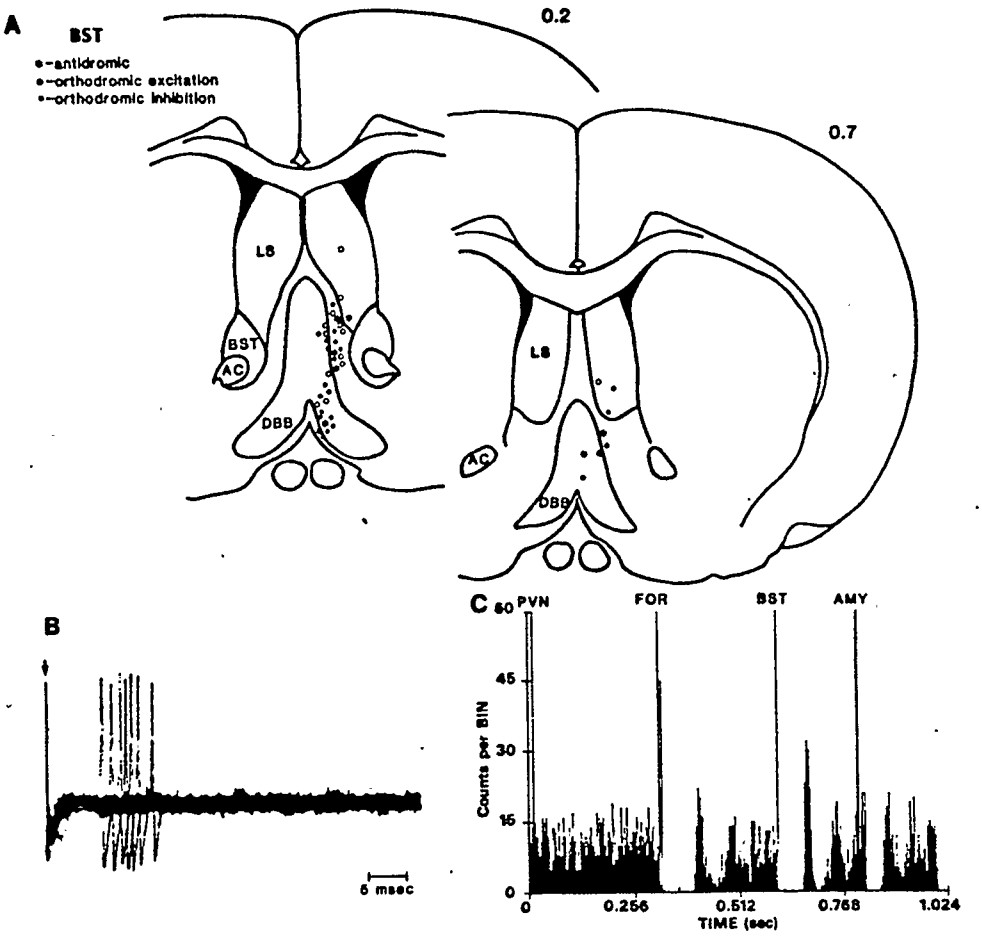

A
PVN
•-antidromic
o-orthodromic excitation
•-orthodromic inhibition
0.2
0.7
LS
BST
AC
DBB
LS
AC
DBB
B
10 msec
C
5 msec

FIG. 2

A. Coronal sections of rat brain 0.2 and 0.7 mm rostral to bregma on which are plotted cells which responded to BST stimulation with antidromic invasion (*), orthodromic excitation (o) or orthodromic inhibition (•). (LS, lateral septum; AC, anterior commissure; DBB, diagonal band of Broca).

B. Oscilloscope trace of 10 superimposed sweeps demonstrating orthodromic excitation of a unit in the VSA following electrical stimulation of the BST at 1.0 Hz. Arrow denotes stimulus artifact.

C. Post stimulus histogram of 1.0 sec duration illustrating the response of a VSA cell to PVN, fornix (FOR), bed nucleus of the stria terminalis (BST) and amygdala (AMY) stimulation.


A
BST
•-antidromic
•-orthodromic excitation
•-orthodromic inhibition
0.2
0.7
LS
BST
AC
DBB
B
5 msec
C
PVN
FOR
BST
AMY
60
45
30
15
0
Counts per BIN
0
0.256
0.512
0.768
1.024
TIME (sec)

orthodromic activation from stimulation of the BST also responded to stimulation of the PVN. Although these two areas are anatomically close to each other, there was no consistent pattern of evoked response observed when stimulating both areas; that is, an excitatory orthodromic input from the BST was observed to be accompanied by either orthodromic excitatory or inhibitory inputs or by antidromic activation from the PVN. Nonetheless, locations of cells which were activated from the BST were similar to those cells which responded to PVN stimulation.

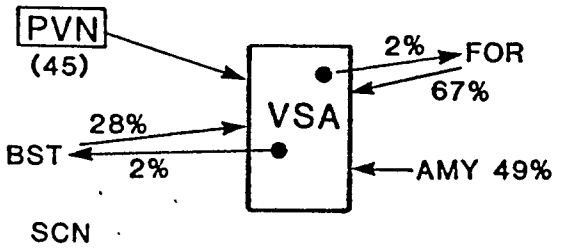
Sixty-one neurons were tested for their response to SCN stimulation of which 17 demonstrated orthodromically driven responses. It is notable that the majority of the responsive neurons were inhibited by stimulation of the SCN. The inhibitory period occurred after a latency of 5 msecs and often persisted for 40 msecs. Whereas, neurons receiving SCN inputs also responded to the BST, convergence of input from the PVN was not found. Again, the orthodromic potentials from stimulation of the SCN to appear to be found generally in the more ventral septal areas where vasopressin induced antipyresis has been shown (Kasting, 1980; Naylor et al, 1985a) and less so in the lateral septal nucleus.

Stimulation of the fornix and amygdala evoked orthodromic responses (either excitatory or inhibitory) in a substantial number of lateral septal and VSA neurons but these did not appear to preferentially innervate any one class of identified neurons. It was common to find a high degree of convergence of afferent input from these areas with that of the PVN, BST or SCN. Figure 3 provides a

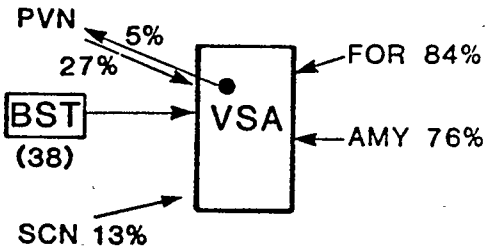
FIG. 3

Summary of connectivity demonstrating interactions between VSA cells responding to PVN (upper left), BST (upper right) and SCN (lower centre) stimulation. The total number of cells in which an orthodromic response (excitatory or inhibitory) could be evoked by stimulation of each area are indicated in brackets. The percentages indicate the number of cells which, in addition to being orthodromically activated by stimulation of the nucleus indicated in the box, were also responsive to stimulation of the various other areas studied.

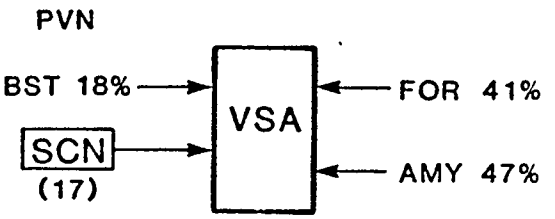
PVN orthodromic profile



BST orthodromic profile



SCN orthodromic profile



summary of the interactions between the various areas stimulated and VSA neurons.

D. Discussion

The demonstration of PVN connections to the lateral septal area is consistent with previous anatomical (Buijs et al, 1978; Conrad and Pfaff, 1976b) and electrophysiological (Pittman et al, 1981) studies. Although a large portion of the neurons antidromically activated by stimulation of the PVN were found in the lateral septum, orthodromic potentials following PVN stimulation were predominantly found in more ventral areas (VSA). The present study appears to be the first electrophysiological study demonstrating PVN and BST projections to the VSA and DBB. Both excitatory and inhibitory responses were observed, suggesting that more than one transmitter (or neuron) is involved in these projections. The involvement of AVP is still uncertain; whereas, immunocytochemical studies have shown simultaneous changes in quantity of immunoreactive AVP in the PVN and septal areas (suggesting a possible connection) lesion and transport studies have not provided convincing evidence for a PVN-lateral septal, VSA vasopressinergic pathway (DeVries and Buijs, 1983).

This data revealing afferents from the BST indicate that this nucleus could provide the AVP in the lateral septum and VSA. In support of this are reports that electrolytic lesions of the BST decrease immunoassayable AVP content of the lateral septum and DBB (DeVries and Buijs, 1983).

The contribution of the SCN to lateral septal AVP innervation has been controversial. Arginine vasopressin-immunoreactive fibers appeared to project from the SCN to the lateral septum (Buijs et al,

1978; Sofroniew and Weindl, 1978), but recent lesion studies indicate that lateral septal AVP content is not reduced by SCN ablation (Hoorneman and Buijs, 1982). This study, as well as previous anatomical studies (Moore, 1983) suggest that the SCN innervates the VSA and lateral septum, although the small size of the nucleus makes it possible that nearby fibres and cell bodies could have been activated as well by the stimulating current. This projection was largely inhibitory in nature, but whether or not AVP is the transmitter responsible for this input is unknown. Recent electrophysiological studies have revealed many excitatory effects of AVP on cellular activity (Joels and Urban, 1982; Muhlethaler et al, 1982; Tiberiis et al, 1983) making it likely that one or more of the other transmitters present in the SCN (Moore, 1983) are involved in these evoked responses.

The present study also reported afferents from the fornix to the septal areas. Joels and Urban (1982) showed that glutamate mediated excitatory input from the fornix to the lateral septum could be enhanced by concurrent iontophoretic application of AVP. They suggested that the observed actions of AVP were postsynaptic in location. In keeping with this possibility are our findings of convergent inputs from the fornix and BST or PVN onto lateral septal and VSA neurons. However, inhibitory convergent inputs were also observed. Such inhibitory interactions are not in support of the observations made by Joels and Urban(1982).

Although immunoreactive AVP containing cell bodies, fibres and terminals have been shown in numerous brain regions, outside of the hypothalamo-neurohypophyseal system, fully described and characterized

central AVP pathways from source to termination remain few. Anatomical data indicates the likelihood of AVP projections from the PVN, BST and SCN to the VSA. This study provides electrophysiological evidence in the rat brain in support of such projections to the VSA where this peptide has been shown to exert antipyretic actions.

III. IDENTIFICATION AND CHARACTERIZATION OF THERMORESPONSIVE NEURONS IN THE VENTRAL SEPTAL AREA

A. Introduction

It is well established that the integrity of the POAH region is essential for normal thermoregulatory functions. Both thermosensitive (neurons responsive to hypothalamic temperatures) and thermoresponsive neurons (those which respond to the activation of cutaneous warm and cold thermoreceptors) have been described in the POAH and are believed to be important components of the neuronal integration of thermal information in this area. Thermosensitive neurons in the POAH may be a target of the endogenous pyrogen molecule responsible for fever (Eisenman, 1969; Wit and Wang, 1968b). The mechanism by which endogenous pyrogen acts to cause an increase in body temperature has not been determined, yet the magnitude of the fever response appears to be modulated as it rarely exceeds 4°C above normal body temperature (Banet, 1979). Evidence has now been presented which implicates the VSA of the basal forebrain as a site where the putative neurotransmitter, AVP acts as an endogenous antipyretic (Cooper et al, 1979). Previous to these observations, the septal area had not been implicated as a primary structure involved in the febrile process. However, the VSA has major connections with the POAH via the medial forebrain bundle (Nauta and Haymaker, 1969). Furthermore, electrophysiological studies have reported both thermosensitive neurons in the septal area (Nakayama et al, 1961) which are pyrogen sensitive (Eisenman, 1969; Wit and Wang, 1968b), and thermoresponsive neurons which are responsive to scrotal skin

temperature (Nakayama et al, 1979). No specific functional role, however, has been ascribed to these population of cells.

Neurons of the VSA which receive afferent input from regions representing possible sources of AVP to the VSA have now been identified in this thesis. These include the PVN and BST which anatomical studies have identified as sources of extrahypothalamic AVP innervation (Buijs et al, 1978; Van Leeuwen and Caffe, 1983). The potential involvement in thermoregulatory processes of neurons in the VSA displaying such connections has not been established. Therefore, the purpose of this study was to identify thermoresponsive neurons of the VSA and to characterize these identified units with respect to their connectivity to the PVN and BST.

B. Experimental Procedures

All experiments were carried out on male Sprague Dawley rats using identical extracellular single unit recording techniques as those previously described. Rats were anaesthetized with urethane and stimulating electrodes were implanted in the PVN, BST, fornix and amygdala. Extracellular recordings were obtained in the VSA as detailed in Chapter II. Body temperature was maintained at 37 °C throughout the experiment and scrotal skin temperature maintained at 25 °C during the search for single unit activity and evaluation of connectivity.

Subsequent to the characterization of an isolated cell's response to stimulation of the various areas, thermoresponsiveness was tested by slowly altering scrotal skin temperature between 15 °C and 45 °C with a water perfused copper thermode held lightly on the shaved scrotum. Although these temperatures are within the range used by previous

investigators (Nakayama et al, 1979; Davies et al, 1985; Hellon et al, 1975) and are not considered to be noxious (Darian-Smith, 1984) this was verified by using blood pressure increases as an indication of painful stimulation. During experiments on four animals a femoral arterial catheter was inserted and connected to a Statham pressure transducer and a Gould pen recorder for monitoring blood pressure. The blood pressure and neuronal responses were observed during the painful stimulation of pinching the foot or scrotum with tissue forceps and compared to the blood pressure and neuronal responses while altering scrotal skin temperature between 5 °C and 45 °C.

In two other experiments the effect of altering scrotal skin temperature on the temperature of the brain was examined to verify that thermoresponsive neurons were activated by peripheral thermal input from the scrotum and were not also being influenced by changes in the temperature of the brain. For these experiments a YSI brain thermistor was lowered into the right side of the brain so that it rested in the region of the right VSA. The temperature of the right VSA was then monitored throughout scrotal skin temperature changes, while single unit recordings were made from neurons in the left VSA. Integrated activity of each isolated single unit was monitored on a Gould paper recorder throughout a minimum of two complete cycles of scrotal temperature changes. In accordance with earlier observations (Wit and Wang, 1968a; Hellon and Mitchell, 1975), a cell was classified as thermoresponsive if a consistent change of greater than 50% in firing rate was observed in response to either heating or cooling of the scrotum.

At the termination of an electrode penetration, the tracts and all sites of interest were marked by iontophoresis of pontamine sky blue dye. Histological verification was then made of all stimulating and recording sites.

C. Results

i) Thermal Studies

Of 140 isolated units in the VSA tested for their responses to scrotal skin temperature changes, 37 (26%) demonstrated activity changes corresponding to alterations in scrotal skin temperature. Figure 4 shows that responsive cells were reactive to changes in scrotal skin temperature and that the temperature of the brain did not change during the experimental procedure. Figure 5 shows both thermoresponsive and nonthermoresponsive cells in the VSA that were not responsive to noxious stimulation which caused increases in blood pressure. This indicates that the temperature range of scrotal skin temperature used in these experiments is probably not within the noxious range and that the thermoresponsive cells observed in this study were not also responsive to noxious stimulation. Figure 6 shows the location of thermoresponsive neurons in the VSA. These cells are found at 0.2 to 0.6 mm anterior to begma in the ventral portion of the lateral septum, medial to the anterior commissure and just lateral to and within the diagonal bands of Broca. Twenty three responsive units displayed increases in firing rate in response to increases in scrotal skin temperature and were classified as warm responsive (Figure 7A). Of these, 6 exhibited a switching response or threshold for activation such that activity changes occurred suddenly at scrotal skin temperatures over 25°C. Upon repeated testing the threshold for a

FIG. 4

A. Rate meter recording of a warm responsive cell in the VSA (top) which demonstrated an increased firing rate in response to an increase in scrotal skin temperature (centre). The temperature of the brain remained constant at 35°C throughout the changes in scrotal skin temperature (bottom trace A and B).

B. Rate meter recording of a nonthermoresponsive cell (top) which demonstrated a constant firing rate throughout scrotal skin temperature changes (centre).

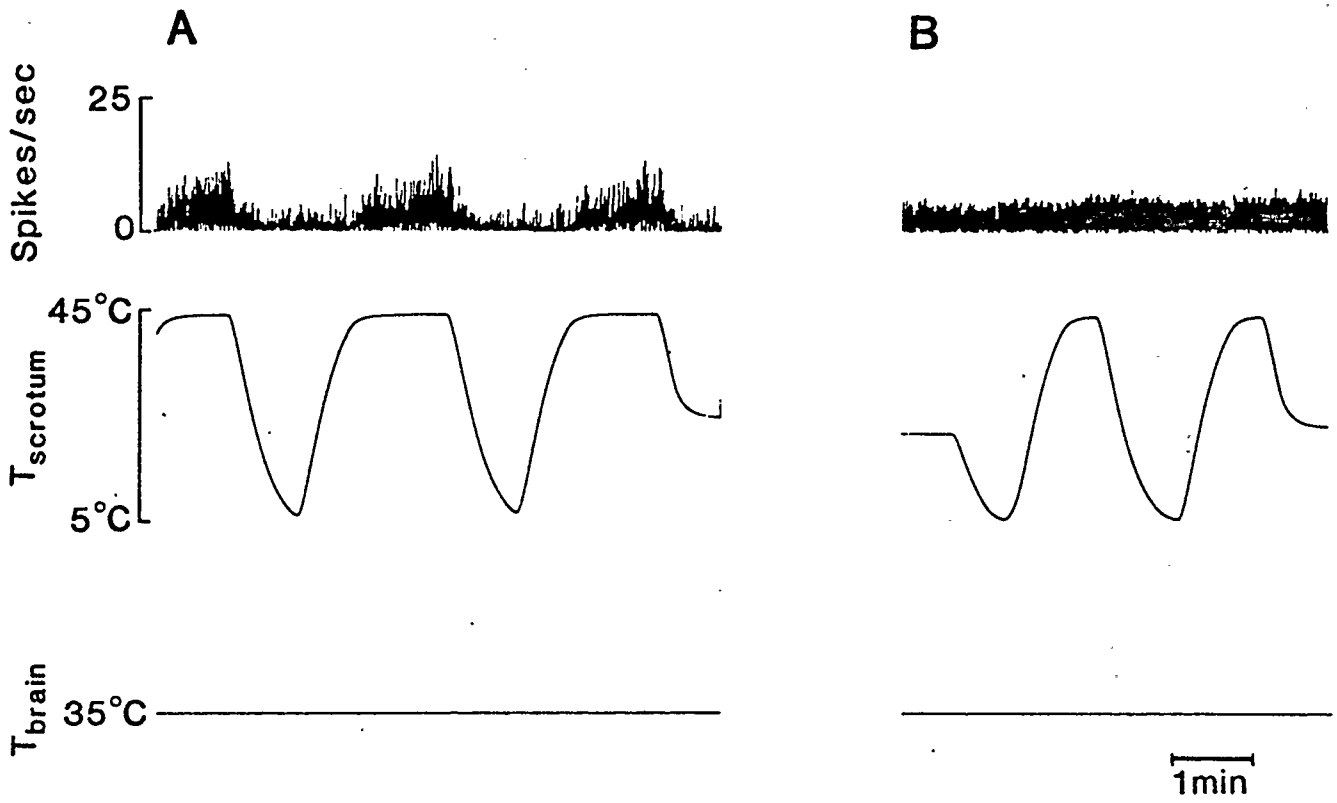

A
B
Spikes/sec
25
0
45°C
$T_{scrotum}$
5°C
T_{brain}
35°C
1min

FIG. 5

A. Rate meter recording of a nonthermoresponsive cell (top) which maintained a constant rate of firing throughout changes in scrotal skin temperature (centre). Blood pressure recordings (lower trace) also remained stable throughout the same thermal stimulation. This suggested that noxious thermal stimulation was not used.

B. Rate meter recording of a warm responsive cell (top) which increased firing when scrotal skin temperature was increased (centre). No increases in blood pressure (bottom) corresponding to increased skin temperature or firing rate were observed.

C. Rate meter recording of a cold responsive cell (top) which increased firing when scrotal skin temperature was decreased (centre). Painful foot pinches (arrows) which caused increases in blood pressure (bottom) did not affect the firing rate of this cell.

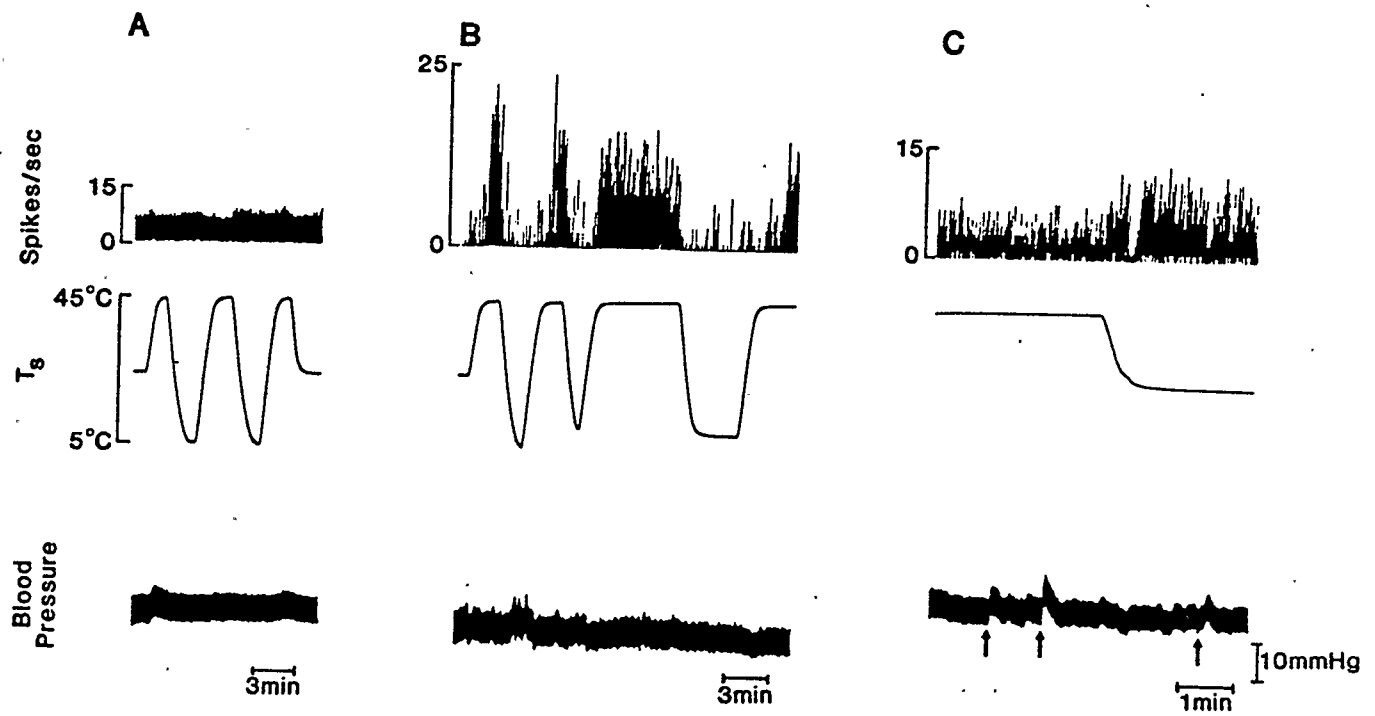

A
B
C
Spikes/sec
15
0
25
0
15
0
45°C
T_s
5°C
Blood Pressure
3min
3min
1min
10mmHg

FIG. 6

Coronal section of the rat brain at 0.2 mm anterior to bregma showing the location of thermoresponsive neurons in the ventral septal area. ● denotes warm responsive, o cold responsive and * other which includes dynamic, biphasic and phasically active thermoresponsive neurons. As the locations of cells were determined histologically to be scattered throughout both the DBB and adjacent tissue, the general location of the cells is described as VSA.

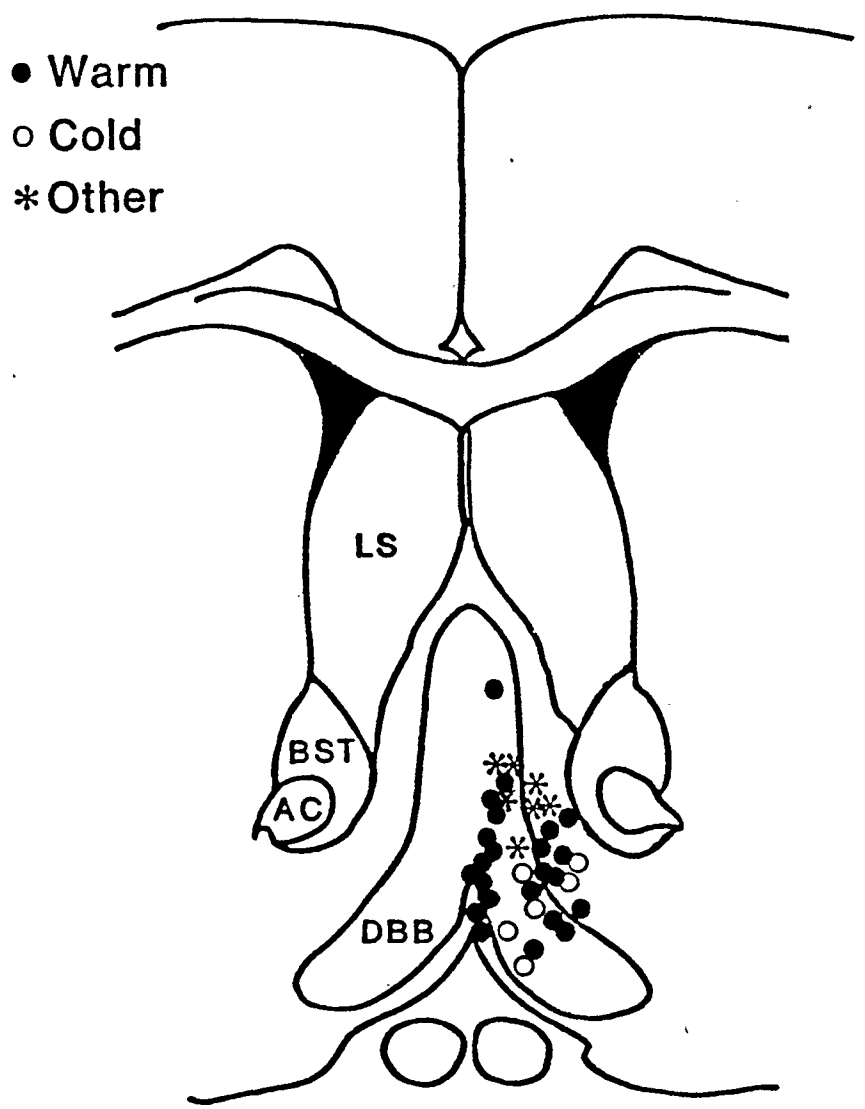
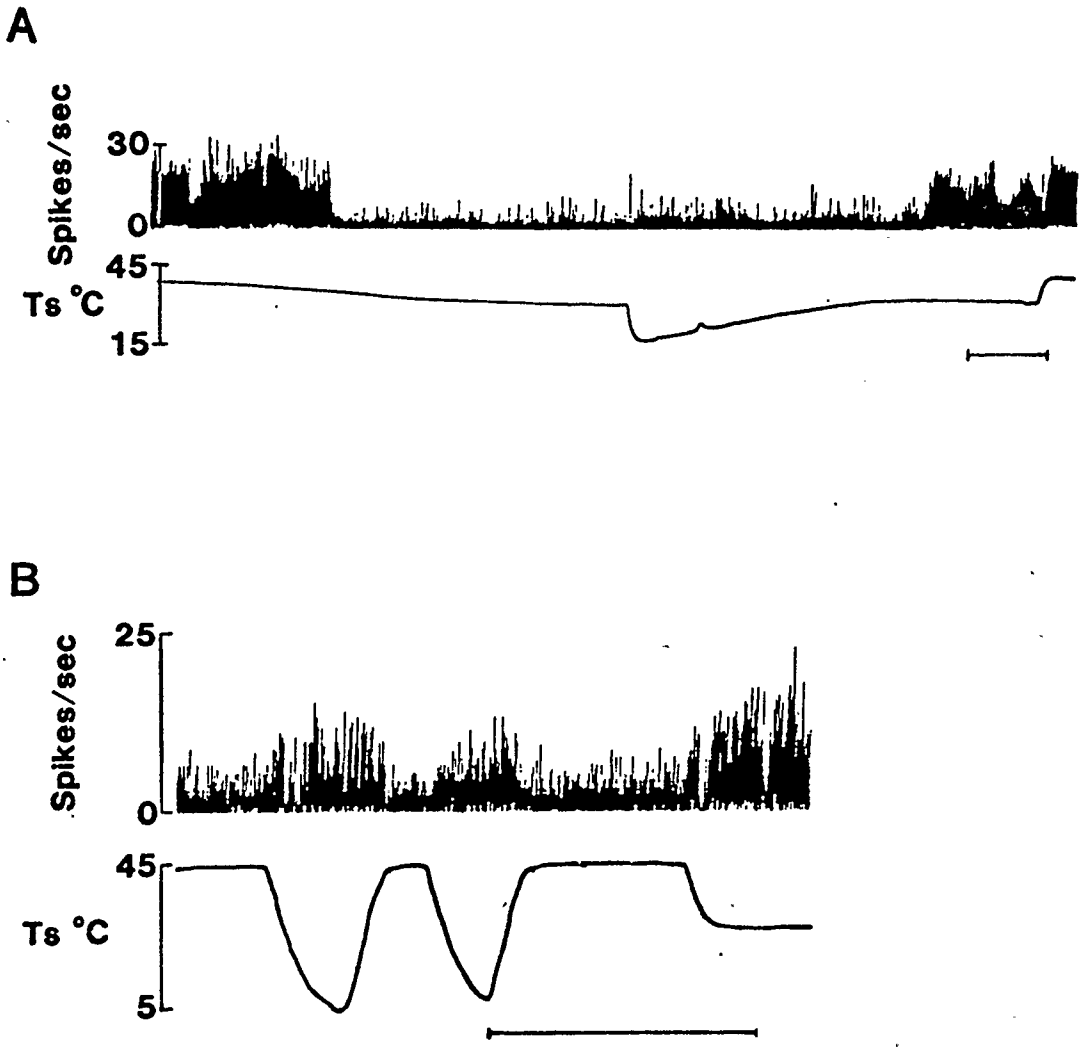

● Warm
o Cold
* Other
LS
BST
AC
DBB

FIG. 7

A. Continuous rate meter record of a warm thermoresponsive VSA cell (upper trace) which demonstrated a reduction in spontaneous activity in response to cooling of the scrotal skin (lower trace).

B. Continuous rate meter record of a cold thermoresponsive VSA cell (upper trace) which demonstrated an increase in spontaneous activity in response to cooling of the scrotal skin (lower trace). Bar mark indicates 5 mins in each case.


A
Spikes/sec
30
0
45
Ts °C
15
B
Spikes/sec
25
0
45
Ts °C
5

particular unit varied less than 2°C. The remaining 17 warm responsive units showed activity changes with phase shift or hysteresis-like properties.

Cold responsive units in which increases in firing rate were observed when scrotal skin temperature was reduced numbered 6 of the 36 responsive cells. Two of these cells displayed thresholds for activation at scrotal skin temperatures of 33°C and 42°C (Fig. 7B) while the remaining four showed the slower hysteresis response (Fig. 9).

In addition, three other response patterns were observed in neurons of the VSA which were not readily identifiable as deriving from warm or cold thermoreceptors and therefore could not be classified as standard warm or cold responsiveness (Fig. 8). One unit displayed a dynamic response which was defined as an activity change in response to dynamic changes in the temperature of the scrotal skin. In this case, abrupt decreases in firing were observed only during the falling phase of scrotal skin temperature changes (Fig. 8A). This is unlike the warm (cold) responsive unit which maintains it's increased firing rate at any scrotal skin temperature above (below) the temperature at which it is activated whether this temperature is increased, decreased or remains static.

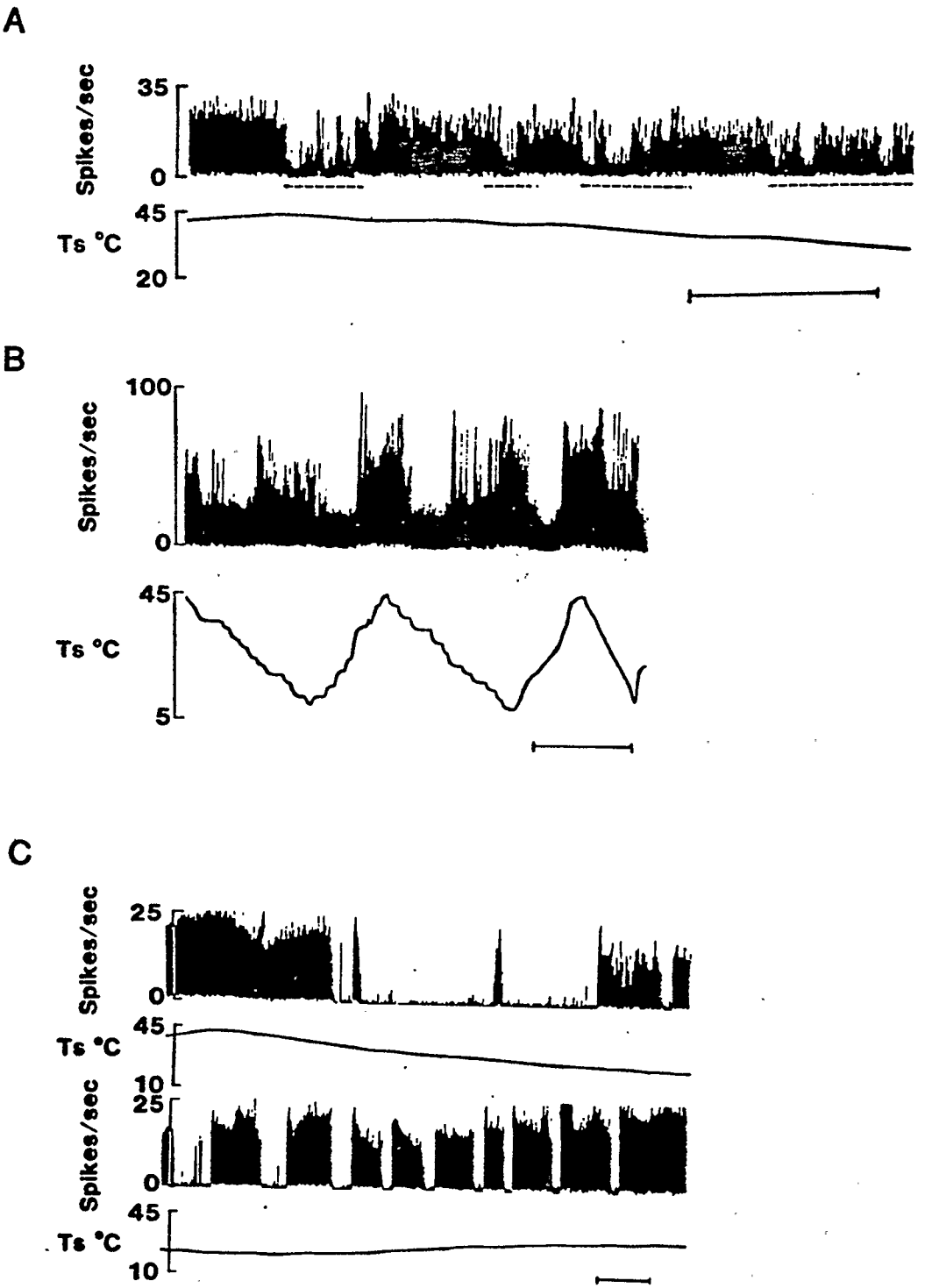
Four other units showed biphasic responses in which increases in firing rate occurred when scrotal skin was either warmed or cooled. The lowest firing rate was observed in the intermediate temperature range between 35 and 25°C when reducing and 15 and 30°C when raising scrotal skin temperature (Fig. 8B).

FIG. 8

A. Continuous rate meter record of a dynamic thermoresponsive VSA cell (upper trace) which demonstrated a reduction in spontaneous activity in response to cooling of the scrotal skin (lower trace). This activity reduction occured only when the temperature was changing (dotted lines) and did not occur when scrotal skin was warmed.

B. Continuous rate meter recording of a biphasic thermoresponsive VSA cell (upper trace) in which increases in spontaneous activity occurred when the scrotal skin was warmed and cooled (lower trace). Reduced spontaneous activity occurred in the intermediate temperature ranges.

C. A continuous rate meter recording of a phasically active thermoresponsive cell in the VSA (upper trace) which demonstrated a change from a rapid firing rate to a phasic activity pattern in response to cooling of the scrotal skin temperature (lower trace). The rapid firing rate resumed when the scrotal skin was warmed. Bar mark indicates 5 mins in each case.


A
Spikes/sec
35
0
Ts °C
45
20
B
Spikes/sec
100
0
Ts °C
45
5
C
Spikes/sec
25
0
Ts °C
45
10
Spikes/sec
25
0
Ts °C
45
10

Three other units also displayed activity changes resulting from scrotal skin temperature alterations which were similar to the biphasic response. When tested for thermoresponsiveness, one unit displayed a biphasic response but later showed a phasic activity pattern which could be induced by lowering scrotal skin temperature (Fig. 11). Two other neurons displayed maximal firing rates at the higher and lower scrotal skin temperatures but were induced into a phasic firing pattern in the intermediate temperature ranges (Fig. 8C, Fig. 10). The phasic activity of these three neurons consisted of bursts of firing of 30 sec. to 5 minutes duration followed by abrupt periods of silence lasting between 30 sec. and 12 minutes.

ii) Connectivity Studies

In the present study, identified thermoresponsive units were also studied for their possible connections to the PVN, BST, fornix and amygdala. All areas studied showed only afferent connections to the VSA with the exception of one incidence in which an efferent projection to the PVN was observed. Table 1 summarizes the results of the connectivity studies.

Stimulation of the PVN evoked responses in 12 of 20 thermoresponsive units. Orthodromic inhibitory responses were observed in 9 neurons (4 warm responsive, 2 cold responsive, 1 phasically active and 2 biphasic) indicating afferent input (see Fig. 10). One responsive unit was antidromically activated by stimulation of the PVN.

Twenty one thermoresponsive units were tested for their response to stimulation of the BST of which 15 responded. Orthodromic responses were observed in all cases. The BST appeared to provide a

Site of Stimulation	Responses to Stimulation			Thermoresponsiveness
	Orthodromic Inhibition	Orthodromic Excitation	Antidromic	
PVN (60.0%)	4 2 3 (1 phasic 2 biphasic)		1	warm cold other
BST (71.4%)	7 1 2 (1 phasic 1 biphasic)	1 2 2 (biphasic)		warm cold other
Fornix (73.5%)	13 3 6 (1 dynamic 3 biphasic 2 phasic)	2 1		warm cold other
Amygdala (93.0%)	11 2 4 (1 dynamic 1 phasic 2 biphasic)	8 2		warm cold other

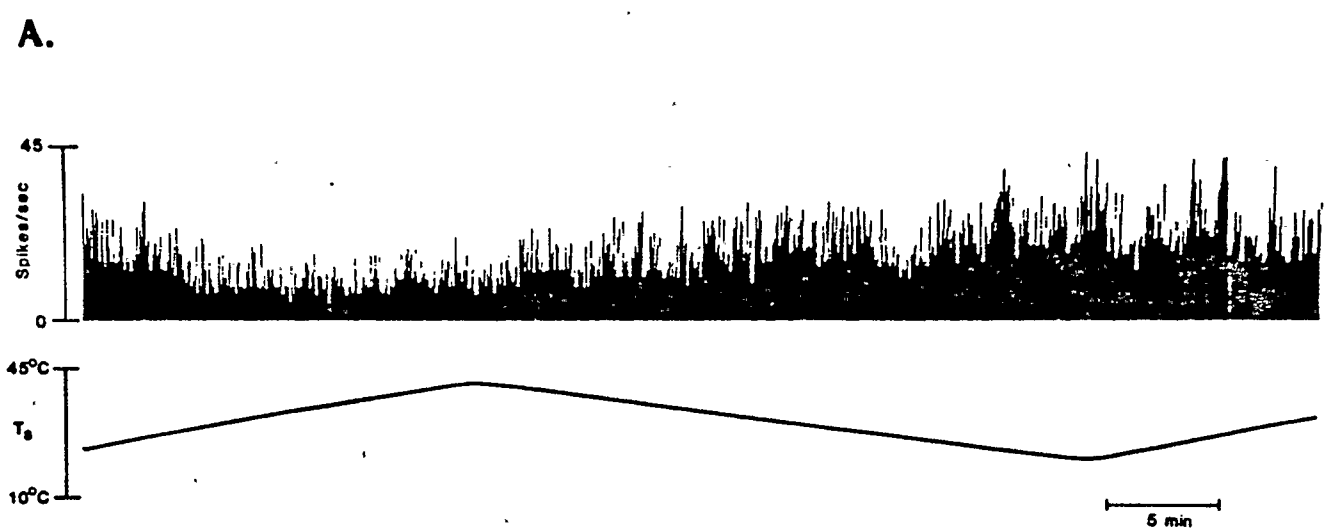
Table 1: Summary of responses elicited in VSA thermoresponsive units by electrical stimulation of the PVN, BST, fornix & amygdala. The percentage is a measure of the thermoresponsive cells studied which responded to stimulation of that particular area. See text for further explanation. PVN, paraventricular nucleus; BST, bed nucleus of the stria terminalis.

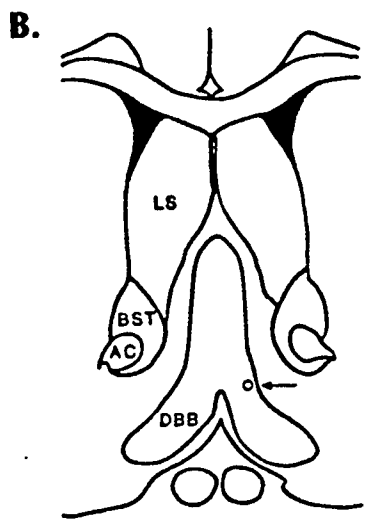
FIG. 9

A. Continuous rate meter recording of a cold thermoresponsive unit in the VSA (upper trace) which demonstrated an increased activity to cooling of the scrotal skin (lower trace). This cell is an example of a thermoresponsive unit showing a slow hysteris response to scrotal skin temperature changes.

B. Coronal section of the rat brain at 0.2 mm anterior to bregma showing the location of this cell in the VSA.

C. A schematic representation of the connectivity of this cell indicating that orthodromic inhibition was observed from stimulation of the BST, PVN, fornix and amygdala.


A.
45
Spikes/sec
0
45°C
T_a
10°C
5 min


B.
LS
BST
AC
DBB

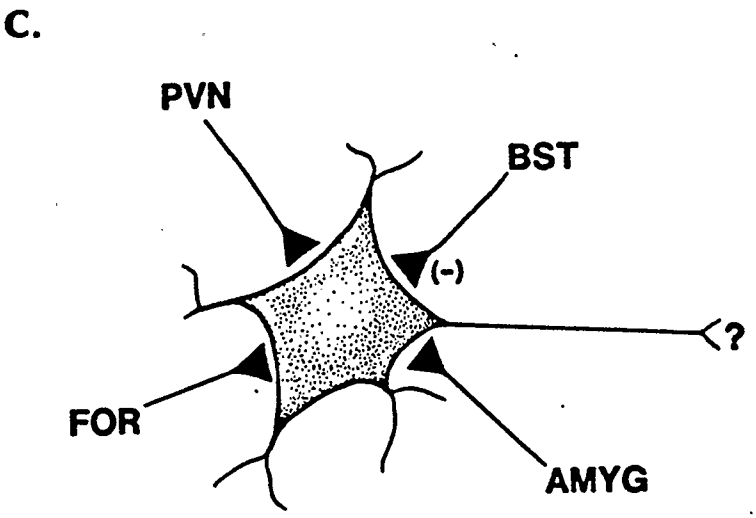

C.
PVN
BST
(-)
?
FOR
AMYG

FIG. 10

A. Rate meter recording of a phasically active thermoresponsive cell in the VSA (upper trace). Note that as the scrotal skin temperature was reduced (lower trace) this cell adopted a phasic activity pattern. This phasic pattern only occured at the intermediate temperature ranges with rapid firing occuring at the warm and cold extremes.

B. Coronal section of the rat brain at 0.2 mm anterior to bregma showing the location of this cell in the VSA.

C. A post stimulus histogram of 1 sec duration demonstrating orthodromic inhibition from the PVN and a slight orthodromic excitation from the fornix.

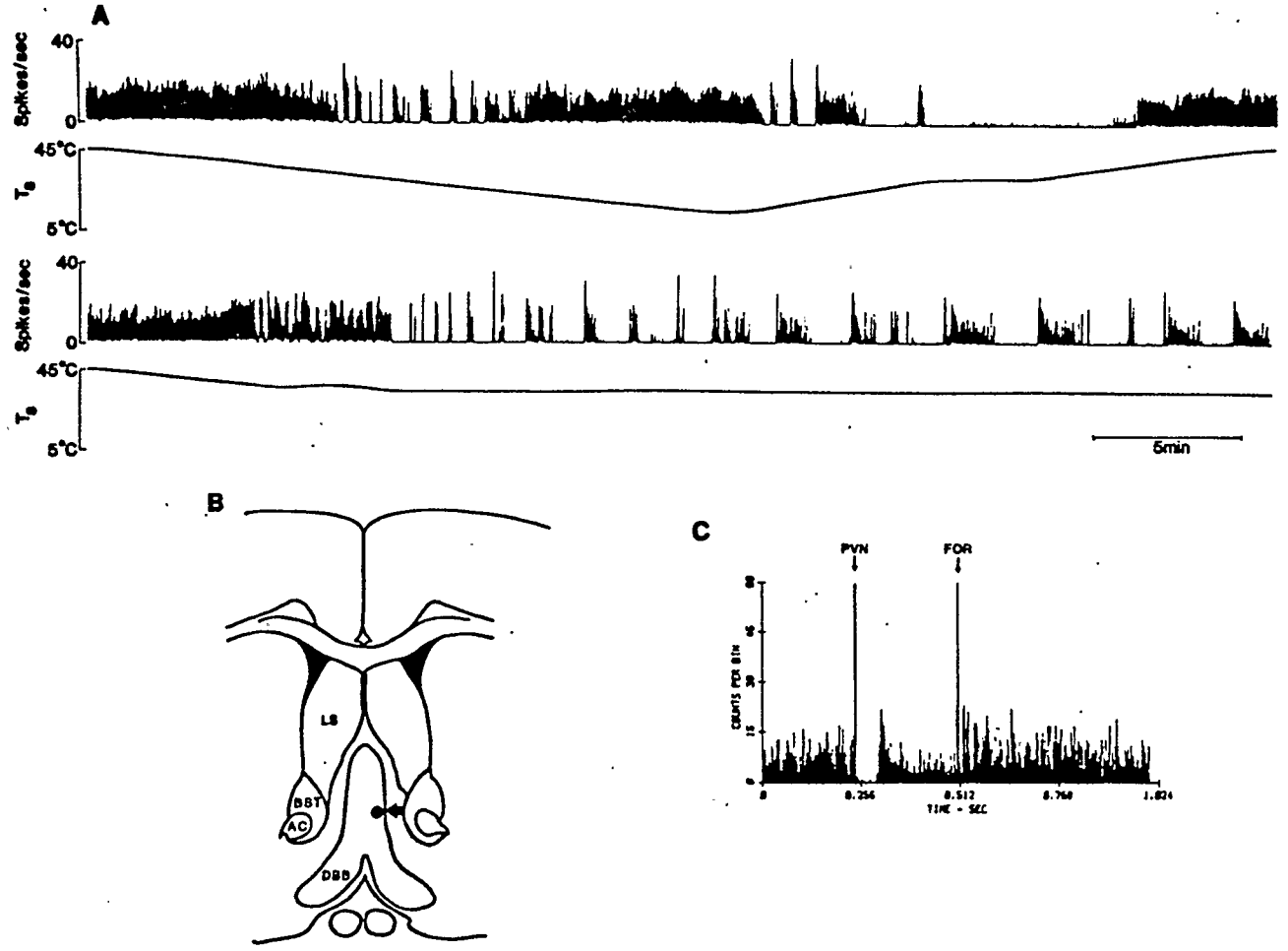
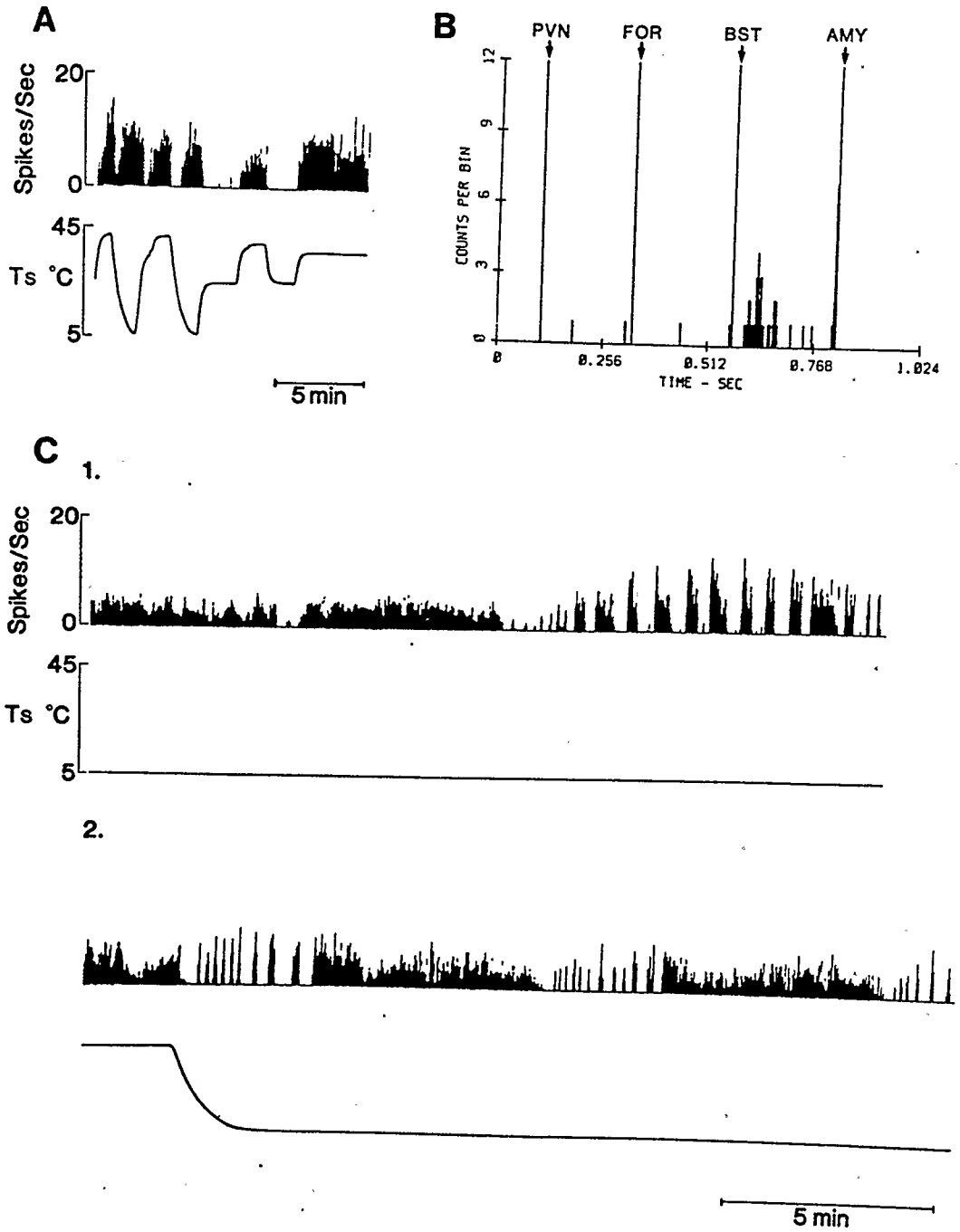

A
Spikes/sec
40
0
45°C
T_s
5°C
Spikes/sec
40
0
45°C
T_s
5°C
5min
B
LS
BST
AC
DBB
C
PVN
FOR
COUNTS PER BIN
0
15
30
45
60
0
0.256
0.512
0.768
1.024
TIME - SEC

Fig. 11

A. Continuous rate meter recording of a spontaneously active VSA neuron displaying a biphasic response (upper) to heating and cooling of the scrotal skin (lower).

B. Post stimulus histogram of 1 sec. duration illustrating a delayed orthodromic excitatory response from electrical stimulation of the BST.

C. Continuous rate meter recording demonstrating the phasic activity pattern of this cell. Note that as scrotal skin temperature was reduced (bottom panel, lower trace) this cell displayed phasic firing activity (bottom panel, upper trace).


A
Spikes/Sec
20
0
45
Ts °C
5
5 min
B
PVN
FOR
BST
AMY
COUNTS PER BIN
12
9
6
3
0
0
0.256
0.512
0.768
1.024
TIME - SEC
C
1.
Spikes/Sec
20
0
45
Ts °C
5
2.
5 min

relatively selective innervation of the warm and cold responsive neurons in the VSA with orthodromic inhibition predominantly in warm responsive neurons and excitation predominantly in cold responsive cells. Biphasic and phasically active neurons were also innervated by the BST.

Stimulation of the fornix resulted in orthodromically evoked responses in 25 of the 34 thermoresponsive units studied. Warm responsive units comprised 15 of these cells of which 13 were orthodromically inhibited. In addition, 9 other thermoresponsive cells were inhibited by afferent input from the fornix (3 cold responsive, 1 dynamic, 3 biphasic and 2 phasically active). Orthodromic excitation occurred in only 3 thermoresponsive neurons (2 warm and 1 cold responsive).

The amygdala appeared to provide approximately equal inhibitory and excitatory input to thermoresponsive units in the VSA. Of 30 tested, 28 were observed to be orthodromically driven by the amygdala. Nineteen warm responsive units showed evoked responses to stimulation of the amygdala. Eleven of these units were inhibited and 8 orthodromically excited. Of the 4 cold responsive units which received afferent input from the amygdala, 2 were inhibited and 2 orthodromically excited. In addition, 4 other thermoresponsive neurons (1 phasically active, 1 dynamic, 2 biphasic) were orthodromically inhibited by amygdala stimulation.

Discussion

The identification of thermoresponsive neurons as described in this study is consistent with other reports of neurons in the ventral septal area which are responsive to peripheral temperature stimulation

(Wit and Wang, 1968a; Nakayama, 1979). The switching response observed in 6 warm and 2 cold responsive units in this study has previously been described for hypothalamic neurons assumed to be involved in afferent thermoregulatory pathways (Hellon and Taylor, 1982). This response is thought to be unique to neurons receiving thermal information from the raphe nucleus (Hellon and Taylor, 1982) which has recently been recognized as a relay site for a portion of the cutaneous thermal afferents travelling to the thalamus (Gottschlich et al, 1981; Gottschlich and Werner, 1985). The phase shift or hysteresis response seen in 17 warm and 4 cold responsive cells may be indicative of interneurons in thermoregulatory pathways which receive considerable synaptic input from other systems (Eisenman, 1982). While both thermosensitive and thermoresponsive neurons have been described in the hypothalamic and septal areas, Nakayama et al (1979) reported that thermosensitive neurons of the POAH and septal region (including the VSA) were also induced to alter their firing rate by changes in scrotal skin temperature. It would therefore appear that thermal afferent information from the scrotum is integrated with information from other brain areas and existing thermodetectors in this region of the brain. The functional significance of this is not yet clear. However, the 3.8 to 1 ratio of warm to cold thermoresponsive neurons reported in this study suggests a region which may be concerned primarily with heat loss mechanisms (Eisenman, 1969). Such an observation is compatible with the evidence indicating that this region is involved in antipyresis (Cooper et al, 1979).

In addition to the warm and cold responsive neurons described in the VSA, thermoresponsive units with dynamic, biphasic and phasic responses to changes in scrotal skin temperature were also identified. Dynamic responses to scrotal skin temperature changes in preoptic hypothalamic neurons have been reported by Nakayama et al (1983). Although the function of this type of neuron is not known these authors speculated that this response is specific for warm receptor input from the scrotum and may serve to detect the direction of ambient temperature changes.

The firing response pattern we refer to as biphasic is similar to the "paradoxical" discharges previously described in cutaneous cold thermoreceptors and cold sensitive neurons in the spinal cord (Hensel, 1981). This study appears to be the first report of a brain thermoresponsive unit demonstrating such a biphasic response pattern to peripheral thermal stimulation. It may be, therefore, that the biphasic thermoresponsive neurons in the study are neurons which receive input from cutaneous cold thermodetectors and/or cold thermoreceptors in the spinal cord which exhibit paradoxical discharges.

The phasic pattern of firing observed in three thermoresponsive units is characteristic of vasopressin secreting neurons of the hypothalamo-neurohypophysial system (Wakerly et al, 1975). However, AVP containing neurons terminating in the neurohypophysis have only been identified in the PVN, supraoptic nucleus and identified accessory neuronal population and this pattern of firing activity has not been reported for mammalian extrahypothalamic vasopressin neurons. Since anatomical studies suggest that, of these sources of AVP for the

hypothalamo-neurohypophysial system, only the PVN may project extrahypothalamically to the septal area (Sofroniew, 1985), it is tempting to speculate that these phasically firing thermoresponsive cells in the VSA may be receiving inputs from AVP containing neurons of the PVN. In keeping with this possibility is the fact that in this study evidence for orthodromic connections between such phasically active VSA neurons and the PVN is also provided. The fact that this phasic activity pattern was only evident over certain temperature ranges suggests either that other afferent input to these cells 'gated' the PVN afferents or that AVP cells themselves are thermoresponsive. In support of the latter possibility are the observations of Matsumura et al (1983) who described antidromically identified phasic neurosecretory neurons in the PVN which were responsive to both increases and decreases of POAH temperature. Since plasma AVP concentrations increase with preoptic warming (Szczepanska-Sadowska, 1974), the response of phasically bursting warm responsive neurons in the PVN may be related to these peripheral changes in AVP.

Information as to some of the other connections of neurons in the VSA which receive peripheral thermal input is now provided. The observation that, of those tested, approximately 74% of all thermoresponsive units in the VSA showed orthodromic responses (88% inhibitory) to stimulation of the fornix indicates a major influence of the hippocampus on these neurons. Other studies have shown that thermosensitive units of the septal region reduce both firing rate (Hori et al, 1982; Osaka et al, 1984) and thermosensitivity (Osaka et al, 1984) in response to stimulation of the hippocampus. The role of the hippocampus in thermoregulation is not clear; however, the

hippocampal region has been shown to respond with graded changes in electrical activity to hypothalamic and skin thermal stimulation (Horowitz et al, 1974; Horowitz and Saleh, 1980).

The amygdala has also been implicated in the integration of peripheral afferent information projecting to limbic regions (Gloor, 1975). Although the functional significance of this area in thermoregulation is not known, evidence for the amygdala as a component of an endogenous AVP antipyretic system is provided by Zeisberger et al (1981) and Merker et al (1980) who reported increased immunoreactive AVP in fibres of the septal area and amygdala in guinea pigs which did not demonstrate a fever when given exogenous pyrogen. This study showing 93% of identified thermoresponsive neurons in the VSA receiving afferent input from the amygdala suggests that this region is somehow involved in processing afferent thermal information to the VSA.

Evidence for a possible role for AVP in thermoregulatory processes of the VSA has also been obtained. In addition to the identification of the phasically active thermoresponsive neuron previously discussed, 75% of the neurons in the VSA which responded to peripheral thermal stimulation also received afferents from potential sources of AVP, namely the PVN and BST. It may be of interest that input from the PVN was largely inhibitory, whereas in the previous chapter of this thesis approximately equal inhibitory and excitatory orthodromic responses in unidentified neurons following stimulation of both the PVN and BST were observed. It may be, therefore, that the inhibitory input from the PVN observed in this study is associated preferentially with thermoresponsive units. The BST, on the other

hand, appeared to orthodromically excite primarily cold responsive units and inhibit warm responsive units. The physiological significance of the distribution of BST inputs to thermoresponsive neurons is not known but it may be somehow related to the known differential action of pyrogens on warm and cold sensitive neurons (Eisenman, 1969; Hori et al, 1984; Wit and Wang, 1968b).

Further evidence has been presented in this study in support of AVP acting as an endogenous antipyretic in the VSA by identifying neurons in this area that are involved in thermoregulatory pathways and are also responsive to areas representing potential sources of AVP to the VSA. The mechanism of action of AVP in this area, however, remains elusive.

IV. NEUROMODULATORY ACTIONS OF IONTOPHORETICALLY APPLIED AVP ON VSA NEURONS

A. Introduction

Iontophoretic studies in anaesthetized rats have revealed a number of different actions of AVP on central neurons. Direct excitatory effects of this peptide on neurons in the locus coeruleus (Olpe and Baltier, 1981) have been reported and in the spinal cord an inhibitory response to AVP iontophoretic application on sympathetic ganglion neurons was observed (Gilby, 1982). In the lateral septum potential modulatory actions of AVP have also been reported. Although excitatory effects of AVP on the spontaneous activity of lateral septal neurons was observed in 30% of the cells studied by Joels and Urban (1984), iontophoresis of AVP has shown this peptide to predominantly interact with other neurotransmitter substances. AVP application has been shown in different laboratories to either enhance or reduce the response to glutamate and other excitatory amino acids (Joels and Urban, 1984; Marchand and Hagino, 1982) and to affect monoamine induced responses in the lateral septum (Joels and Urban, 1985). No indication of the endogenous source of AVP was given in these studies.

A role for AVP as a neurotransmitter in the ventral septal area is strongly suggested by the demonstration of a physiological function (antipyresis) and by the identification in this thesis of afferent projections from potential sources of AVP (the PVN and BST) to VSA neurons. However, the effects of AVP on neuronal activity in the VSA have yet to be demonstrated. Therefore, iontophoretic techniques for the application of AVP and other neurotransmitters have been utilized

to determine if AVP may act alone or interact with other neurotransmitters to alter neuronal activity in the VSA. In addition, VSA cells responsive to AVP were studied for their connectivity to the PVN and BST and characterized with respect to their thermoresponsiveness to provide further evidence in support of a role for AVP in the VSA as a neurotransmitter involved in thermoregulatory processes.

B. Experimental Procedures

Male Sprague Dawley rats were anaesthetized with urethane and implanted with bipolar stimulating electrodes in the PVN, BST, fornix and amygdala. Single unit extracellular recordings, connectivity analysis and thermoresponsiveness testing were made in the VSA using identical techniques to those previously described. The extracellular recording electrode was glued to a 7 barrelled micropipette (20-30 µm tip separation) used for iontophoretic application of drugs. The micropipette barrels contained the following drug solutions: Arginine^{8} vasopressin (AVP) dissolved in acetic acid and mixed with 165 mM NaCl to 10^{-4} M (pH 4), Na+-glutamate dissolved in 165 mM NaCl to .25 M (pH 7-8), 165 mM saline as a control and 3 M NaCl for automatic current balancing. Following the isolation of a single unit its connectivity to the PVN, BST, fornix and amygdala were studied and thermoresponsiveness determined. On 31 spontaneously active cells, glutamate (an excitatory amino acid) was pulsed with fixed negative current intensity (15-30 nA) at regular intervals (10-15 secs on, 10 secs off) until a stable excitatory response was observed. Subsequently the cell's response to glutamate was tested while AVP was delivered continuously with a 35-190 nA current. A 50% change in the

glutamate response following application of AVP was taken to indicate AVP responsiveness. AVP was also ejected using the same positive current to 11 of the 31 spontaneously active neurons studied. To control for current artifact, all application of drugs was performed using automatic current balancing and saline was ejected with identical current as the AVP which produced a response. Retaining currents for the peptides was 5-10 nA and for glutamate ca 10 nA. At the termination of an electrode penetration all tracts and sites of interest were marked with iontophoresis of pontamine sky blue dye. Histological verification was made of all stimulating and recording sites.

C. Results

Thirty one cells in the VSA were tested for their response to AVP. When applied alone to 11 spontaneously active cells, AVP was without effect on 10 cells while 1 showed a slight excitation. Twenty one of the neurons tested, however, showed a reduction in the glutamate excitation during application of AVP (Figs. 12 and 13). One cell showed an enhanced glutamate excitation to AVP iontophoresis. Dose dependent responses were determined for 8 of the AVP responsive cells (Fig. 13) and full recovery of the glutamate response was observed following termination of AVP application in all cells. Ten of the cells which demonstrated a reduction in glutamate excitation to AVP application also showed an increased background activity during AVP application.

The AVP responsive cells were also studied for their connectivity to potential sources of AVP (PVN and BST) and other areas (fornix and amygdala). The results of these studies are summarized in Table 2.

FIG. 12

Oscillograph records of a spontaneously active neuron in the VSA. In each case the horizontal bar indicates the duration of glutamate ejection. Top trace shows increased neuronal activity evoked by glutamate ejection at -35 nA. Centre trace shows the response of the same cell to glutamate application at -35 nA during AVP application at +100 nA. Note the attenuated response to glutamate and the increased background activity between glutamate applications. Bottom trace shows recovery of the glutamate evoked activity following termination of AVP ejection. Glu, glutamate; AVP, arginine vasopressin.

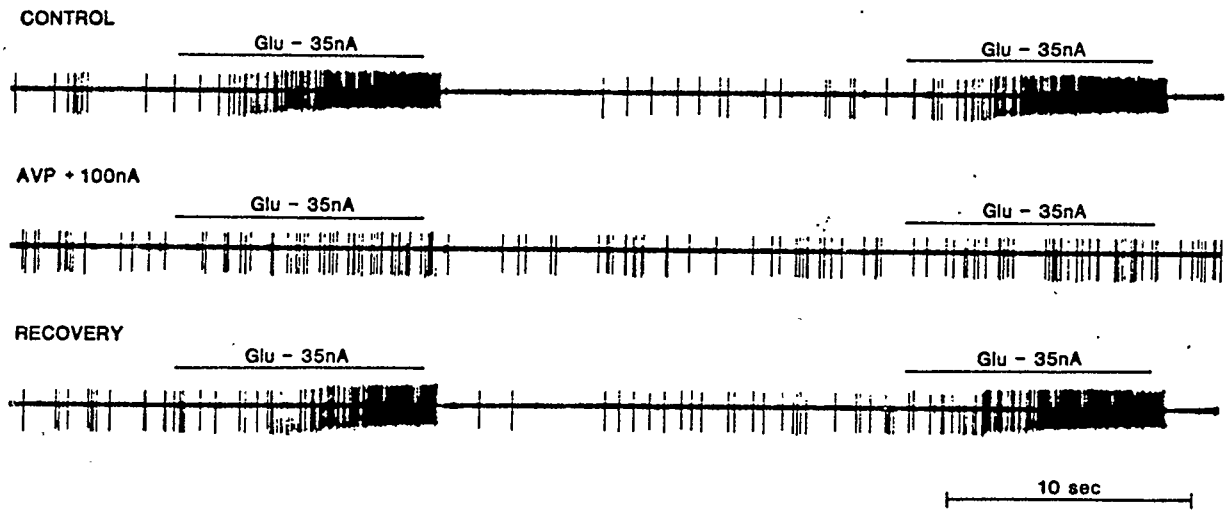
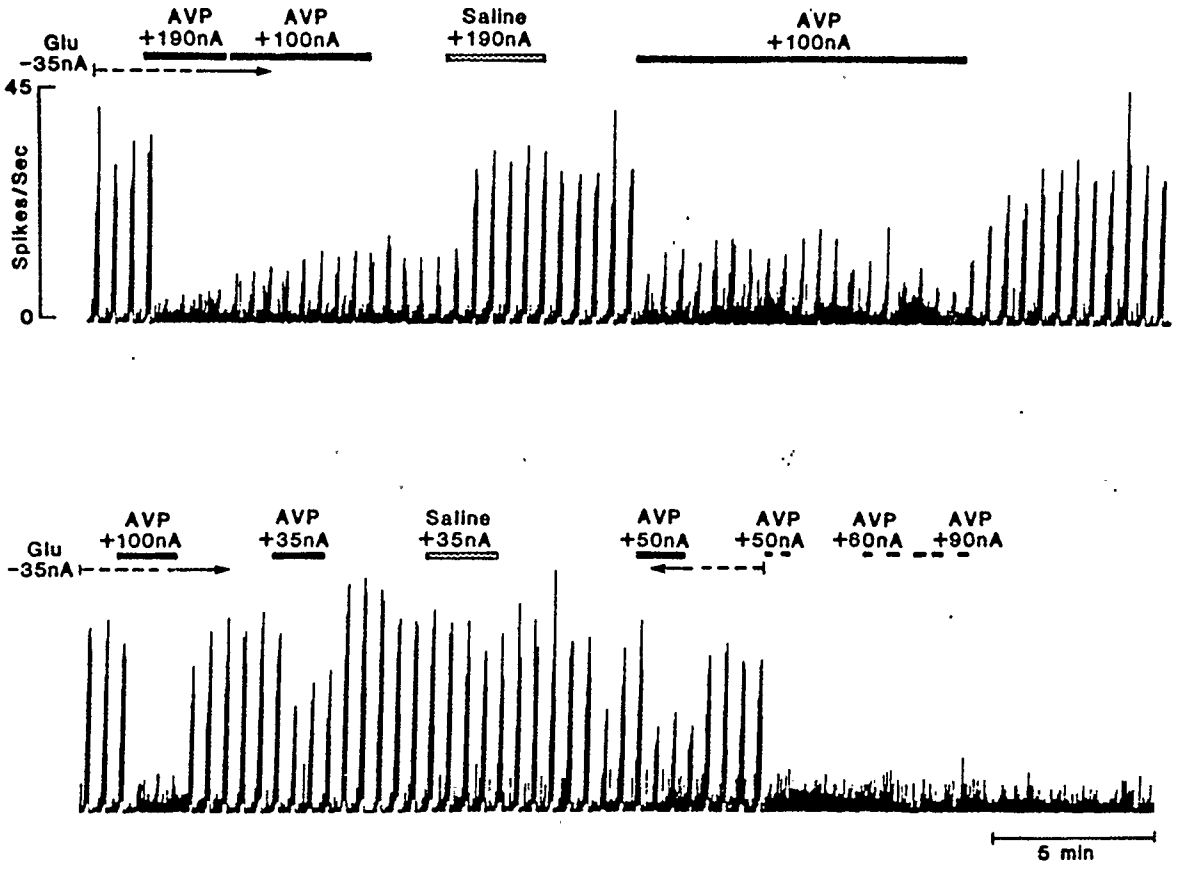

CONTROL
Glu – 35nA
Glu – 35nA
AVP + 100nA
Glu – 35nA
Glu – 35nA
RECOVERY
Glu – 35nA
Glu – 35nA
10 sec

FIG. 13

Rate meter recording of a spontaneously active cell in the VSA. An excitatory response of this cell in response to glutamate ejection in pulses at -35 nA is shown. When AVP was applied (horizontal bars) at currents of +190 nA, +100 nA, +50 nA and +35 nA a dose dependent reduction in the glutamate evoked excitation is demonstrated. Note full recovery following AVP application. Current artifact was controlled for by the application of saline (hatched bars) at +190 nA. Following termination of the glutamate application, AVP ejection at +50 nA, +60 nA and +90 nA had no effect on the spontaneous activity of this cell. Glu, glutamate; AVP, arginine vasopressin.


AVP
+190nA
AVP
+100nA
Saline
+190nA
AVP
+100nA
Glu
-35nA
45
Spikes/Sec
0
AVP
+100nA
AVP
+35nA
Saline
+35nA
AVP
+50nA
AVP
+50nA
AVP
+60nA
AVP
+90nA
Glu
-35nA
5 min

Site of stimulation	Response to Stimulation		
	Orthodromic Inhibition	Orthodromic Excitation	Antidromic
PVN (61%)	5	3	0
BST (72%)	5	3	0
Fornix (70%)	9	3	0
Amygdala (88%)	8	7	0

Table 2: Summary of the responses elicited in VSA AVP responsive units by electrical stimulation of the PVN, BST, fornix and amygdala. The percentage is a measure of the AVP responsive cells studied which responded to stimulation of that particular area.

Eight of the 13 AVP responsive cells tested received afferents from the PVN with orthodromic inhibition being the predominant response. Stimulation of the BST also resulted in 11 of the 13 cells showing primarily orthodromic inhibitory responses. The fornix also provided inhibitory input to AVP responsive neurons in the VSA while the amygdala afferents were equally excitatory and inhibitory. No antidromically identified neurons were observed from stimulation of any of the areas.

Thermoresponsive neurons were also tested for the response to AVP. Of 6 thermoresponsive units studied, 5 of the cells which showed a reduction in glutamate excitation to AVP application also demonstrated thermoresponsiveness to peripheral thermal stimulation (4 warm responsive, 1 cold responsive). Although few in number, the connectivity of these cells was also studied (Table 3). Of 3 neurons tested for their response to stimulation of the PVN, one showed orthodromic inhibition (Fig. 14). Stimulation of the BST evoked 1 orthodromic inhibitory and 2 orthodromic excitatory responses in 4 cells tested. As with the AVP responsive cells, the fornix provided a predominantly inhibitory afferent input to AVP responsive-thermoresponsive cells. The amygdala however, showed excitatory input to all 5 of such cells.

D. Discussion

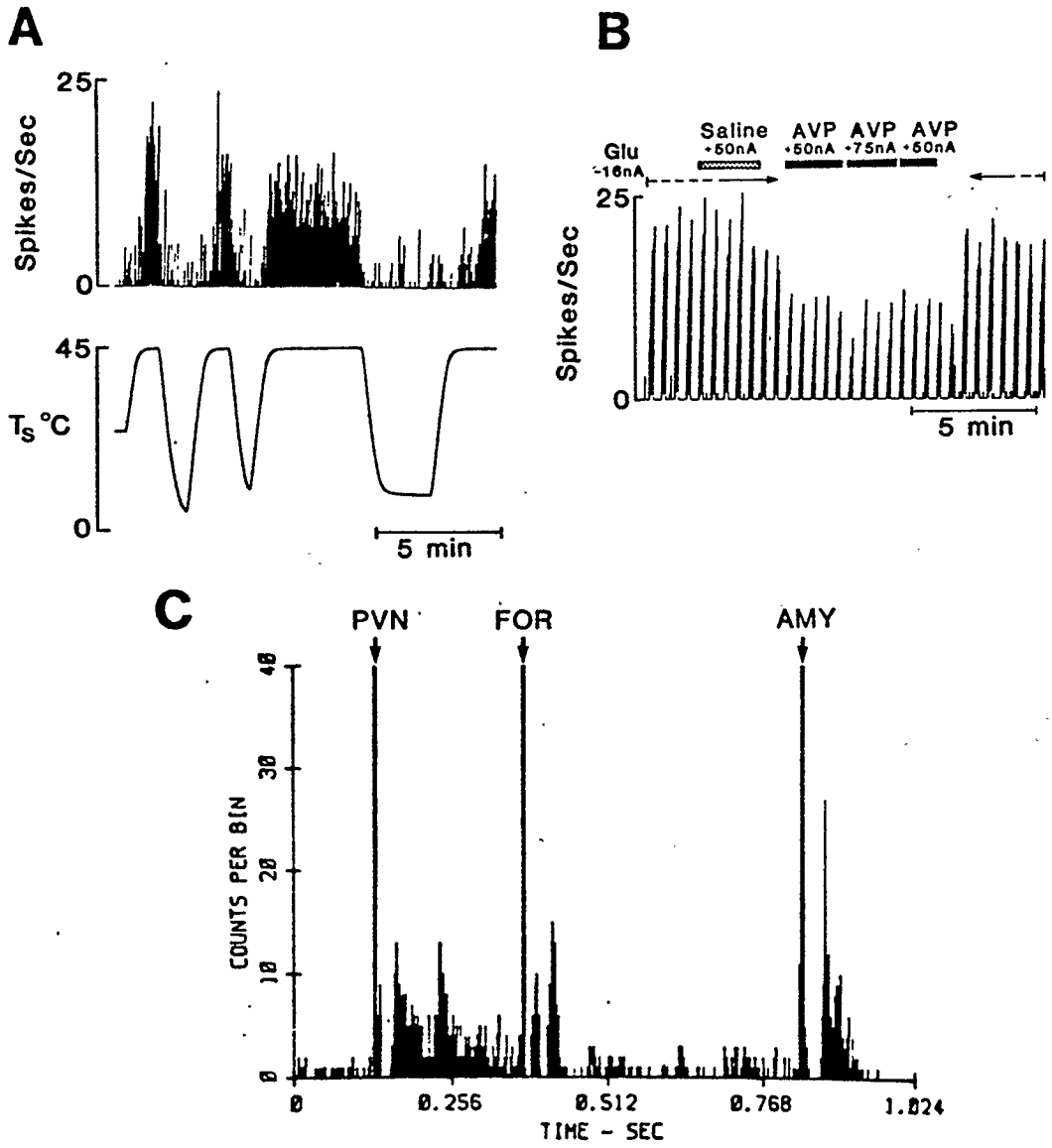
The results of this study suggest that AVP acts as a neuromodulater in the VSA to reduce the excitatory effects of glutamate. These data are in agreement with those of Marchand and Hagino (1982) who showed that AVP iontophoresis suppressed the single unit and field potential responses of lateral septal neurons to

FIG. 14

A. Rate meter recording of a warm responsive cell in the VSA in which increased activity (top trace) occurred when scrotal skin temperature was increased (bottom trace).

B. Rate meter recording of the same cell demonstrating AVP responsiveness. Note that saline ejection at +50 nA had no effect on the glutamate evoked activity of this cell. However, AVP application at +50 nA and +75 nA caused a reduction in the glutamate excitation.

C. Post stimulus histograms of 1 second duration illustrating the response of this warm responsive-AVP responsive cell to stimulation of the PVN, fornix (FOR) and amygdala (AMY). Note the inhibitory response to PVN stimulation which corresponds to the inhibitory action of iontophoretically applied AVP (B).


A
25
Spikes/Sec
0
45
T_S °C
0
5 min
B
Saline
+50nA
AVP
+50nA
AVP
+75nA
AVP
+50nA
Glu
-16nA
25
Spikes/Sec
0
5 min
C
PVN
FOR
AMY
COUNTS PER BIN
0
10
20
30
40
0
0.256
0.512
0.768
1.024
TIME - SEC

Site of Stimulation	Response to Stimulation		
	Orthodromic Inhibition	Orthodromic Excitation	Antidromic
PVN (30%)	1	0	0
BST (75%)	1	2	0
Fornix (80%)	3	1	0
Amygdala (100%)	0	5	0

Table 3: Summary of the responses elicited in VSA AVP responsive - thermoresponsive units by electrical stimulation of the PVN, BST, fornix and amygdala. The percentage is a measure of the AVP responsive - thermoresponsive cells studied which responded to stimulation of that particular area.

glutamate mediated fimbria activation. Joels and Urban (1984) however reported that iontophoresis of AVP enhanced glutamate excitation in lateral septal neurons. While full recovery of the glutamate response was observed in the VSA in this study and by Marchand and Hagino (1982) in the lateral septum, Joels and Urban (1984) reported a prolonged effect of AVP on glutamate excited neurons. It is possible that AVP exerts very different actions on neurons in the VSA and lateral septum, however no explanation was offered for the contrasting observations of Joels and Urban (1984) and Marchand and Hagino (1982).

As yet, no mechanism by which glutamate evoked responses may be modulated by AVP has been proposed. The results of this study indicate a number of mechanisms may be possible to account for the observed effects of AVP in the VSA. Direct postsynaptic effects of AVP on the membrane potential have been reported in supraoptic neurons by Abe et al (1982). Although a depolarization of the membrane potential was observed by these authors, inhibition of spike generation was also reported. This is an unlikely explanation for the observations made in this study since AVP had no effect on the spontaneous activity of 91% of the cells studied. AVP may however act indirectly to decrease input resistance without altering the postsynaptic potential. Such a reduction in input resistance would reduce the voltage change caused by another neurotransmitter (ie. glutamate) and thereby reduce the response of the neuron to that substance. Further studies using intracellular recordings of VSA neurons are required to verify this mechanism.

A more favourable explanation for the observations made in this study may be that AVP has a specific role in modifying a glutamate

activated ionic conductance. Glutamate excitation mediated by an increased conductance to Na+ ions may be blocked by AVP while the effects of other synaptic inputs affecting other ionic conductances remain unaltered. This would involve the interaction of the AVP receptor with the activated glutamate receptor site. Such a mechanisms was proposed by Renaud et al (1979) to explain the effects of thyrotropin releasing hormone on glutamate but not acetylcholine evoked excitation of cortical neurons.

In this study the increased background activity observed in 10 neurons responsive to AVP is also consistent with the observed inhibitory effect of AVP on glutamate excitation. Rapid neuronal firing, as observed with iontophoresis of glutamate, has been shown to activate a Ca^{2}+-dependent K+ conductance in lateral septal as well as hippocampal, motoneuron and hypothalamic magnocellular neurons (Stevens et al, 1984; Andrew and Dudek, 1984; Hotson and Prince, 1980; Krenjevic et al, 1978). An afterhyperpolarization which causes spike inhibition has been attributed to activation of this Ca^{2}+-dependent K+ conductance. AVP acting to reduce the glutamate excitation and therefore the number of spikes evoked by glutamate may diminish an inhibition caused by a Ca^{2}+-dependent K+ conductance. This would result in an increase in the spontaneous background activity as observed in this study during AVP application. However, whether AVP's actions are specific to glutamate excitation and not other excitatory neurotransmitter actions was not determined in these experiments and requires further investigation.

Interactions between neuropeptides and other neurotransmitter substances is often reported. AVP has been shown to alter

catecholamine activity and neuronal responses in the locus coeruleus (Olpe and Baltier, 1981), amygdala (Tanaka et al, 1977; Van Heuven-Nolsen et al, 1984), hippocampus (Church, 1981) and septum (Tanaka et al, 1977; Marchand and Hagino, 1982; Joels and Urban, 1985). In the septum, the action of AVP on glutamate responses is more pronounced than on other excitatory amino acids or catecholamine neurotransmission (Joels and Urban, 1982). The suggestion that AVP alters excitability in the ventral septal area in this study is also supported by reports of AVP acting in this area of the brain to induce severe motor disturbances and convulsive behavior in the rat (Kasting et al, 1981; Naylor et al, 1985).

The studies of the connectivity of AVP responsive neurons also supports a role for AVP as neuromodulator of glutamate activity. Glutamate mediated excitatory input to the septal area from the fornix has been previously reported (Joels and Urban, 1982). In this study, afferents from the fornix to the VSA are also reported, suggesting a possible source of glutamate to this area. Further, those cells which responded to AVP also showed afferent connections with the PVN and BST. The predominantly inhibitory input from these putative endogenous AVP sources also corresponds to the inhibitory action of exogenous AVP. This provides further support for the notion that the endogenous source of AVP in the VSA may be provided by neurons of these two nuclei. Evidence that electrical stimulation of these putative AVP afferents mimic the effects of AVP iontophoresis has yet to be obtained. However, the proposal that endogenous AVP interacts with glutamate in the VSA is supported by the observation of convergent inputs from the fornix and the BST or PVN onto VSA neurons.

With respect to the antipyretic action of AVP in the VSA, it would appear that most of the thermoresponsive neurons in this area are AVP responsive as well. It is not yet possible to relate AVP's neuromodulatory action with the activity of the thermoresponsive neurons during antipyresis since the activity of these neurons during fever and antipyresis is not known. However, it is interesting to note that the AVP responsive- thermoresponsive cells appear to receive proportionally greater innervation from the BST than the PVN. This may indicate that the primary source of AVP to thermoresponsive neurons may be the BST.

Also notable in this study is the inhibitory afferent input from the amygdala on AVP responsive-thermoresponsive neurons. In the previous connectivity studies, the amygdala provided approximately equal inhibitory and excitatory input to both nonthermoresponsive and thermoresponsive neurons in the VSA. The functional significance of this inhibitory input from the amygdala to AVP responsive-thermoresponsive neurons is also not known. However, much evidence exists to implicate this structure as a component of an endogenous antipyretic system. Not only does amygdaloid stimulation cause both increased plasma AVP and a concommitant decrease in brain temperature, but AVP immunoreactivity in both the septum and amygdala also increase during antipyresis (Zeisberger et al, 1981; Merker et al, 1980).

The results of this study therefore suggest that AVP acts as a neuromodulator to reduce glutamate excitation in the VSA. Further support for AVP acting as a modulator in the VSA is provided by the fact that both nonthermoresponsive and thermoresponsive neurons which

respond to AVP also receive afferent input from the PVN and BST. Further work is required to determine how AVP acting to reduce glutamate excitation in the VSA may result in antipyresis.

V. GENERAL DISCUSSION AND CONCLUSIONS

The purpose of the research reported in this thesis was to investigate a possible role for AVP acting as a neurotransmitter or neuromodulator in the VSA where it has previously been shown to be involved in antipyresis. Projections from immunoreactive AVP containing nuclei, the PVN and BST, to the VSA were shown using single unit extracellular recording techniques. This provided evidence for the potential source of endogenous AVP in this area. The demonstration that iontophoretic application of AVP onto ventral septal neurons caused a reduction in glutamate evoked excitation further defined a role for AVP as a neuromodulator. This proposal was supported by the observation that AVP responsive cells in the VSA also received inhibitory afferent innervation from the PVN and BST.

A role for AVP involvement in antipyresis was also supported by evidence presented in this thesis. Neurons responsive to peripheral thermal stimulation of the scrotal skin were identified in the VSA with a frequency similar to that found in the preoptic anterior hypothalamic area. These data indicated that the VSA may be a major component of thermoregulatory processes. That four times more warm responsive than cold responsive neurons were identified also indicated a possible functional role for this area in the control of heat loss mechanisms. AVP involvement in the thermoregulatory pathways of the VSA was suggested by the demonstration of phasically active neurons which were also thermoresponsive. Furthermore, thermoresponsive neurons in this area responded to iontophoretic application of this peptide as well as received afferent input from the PVN and BST.

From the evidence presented in this thesis a number of unanswered questions remain concerning the actions of AVP in the VSA. Now that afferent input from the PVN and BST have been shown to VSA neurons electrophysiologically, anatomical studies are required to confirm the potential involvement of AVP in these projections. The receptor mediating the observed actions of AVP also needs to be determined. To further fulfill the criteria for neurotransmitter status, experiments are also required to show that electrical stimulation of these putative sources of AVP, causing release of this peptide, will mimic the effects of exogenously applied AVP in the VSA. Further, such effects must be eliminated in the presence of AVP receptor antagonists.

The specificity of AVP's actions as a neuromodulator on glutamate evoked activity has not been established. The interaction between AVP and other excitatory neurotransmitters (eg. aspartate, acetylcholine) also requires further investigation. Intracellular studies to determine the neurophysiological mechanisms of AVP actions on the postsynaptic cell in the VSA have yet to be done. Whether a nonspecific ionic conductance is altered or AVP and glutamate receptor interactions occur need to be determined using pharmacological manipulations while recording intracellularly from VSA neurons.

With respect to AVP's involvement in antipyresis, it is interesting to note that the projections from the BST and not the PVN provides selective innervation to warm and cold responsive neurons in the VSA. Since exogenous antipyretics are thought to act by selectively altering the activity of warm and cold thermosensitive neurons in the

brain, this raises the possibility that the AVP involved in antipyresis in the VSA derives from the BST specifically.

Evidence presented in this thesis also indicates that the potential involvement of the amygdala in AVP antipyresis should also be investigated. While immunocytochemical studies reveal AVP immunoreactivity changes in the amygdala during antipyresis the data in this thesis demonstrated that the amygdalar innervation of AVP responsive-thermoresponsive neurons specifically varied from the afferent amygdala innervation of unidentified VSA or VSA thermoresponsive neurons in general.

Also yet to be examined are the effects of AVP on the thermoresponsiveness of VSA neurons or the efferent projections of these cells. It is hoped that further investigation of these unanswered questions may lead to the disclosure of the mechanism of endogenous AVP antipyresis and a further understanding of both normal and abnormal thermoregulatory processes, as well as the basic principles which underlie neuropeptide modulatory actions in the central nervous system.

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