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ARGININE VASOPRESSIN AND THE MEDIAL AMYGDALOID NUCLEUS: A POSSIBLE ROLE IN ENDOGENOUS ANTIPYRESIS

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Arginine Vasopressin and the Medial Amygdaloid Nucleus: A Possible Role in Endogenous Antipyresis" submitted by Paolo Federico in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

Experiments were undertaken to investigate the hypothesis that arginine vasopressin (AVP) might function within the medial amygdaloid nucleus (meA) as an endogenous antipyretic. In the conscious rat, bilateral injection of AVP into the meA evoked drops in core temperature during the plateau phase of interleukin-1 fevers and attenuated hyperthermic responses to the intracerebroventricular injection of prostaglandin E_1 (PGE_1). Further, injection of AVP into the meA of afebrile rats, did not alter core temperature. Thus, AVP appears to be an effective antipyretic when injected into the meA of the rat. In addition, this antipyretic effect is site specific, being localized to the meA and not surrounding regions.

Experiments were also undertaken to determine if AVP, push-pull perfused within the meA or ventral septal area, is antipyretic in the urethane-anaesthetized rat. Perfusion of AVP was found to suppress PGE_1-evoked hyperthermia at both sites, though the meA appeared to be less sensitive to AVP antipyretic action than the ventral septal area.

The central receptor mediating AVP-induced antipyresis in the meA was investigated using AVP analogues directed against V_{1a} and V_2 subtypes of vasopressin receptors. Vasopressin suppressed hyperthermia evoked by intracerebroventricular administration of PGE_1, an effect that was blocked by the V_{1a} antagonist, $d(CH_2)_5Tyr(Me)AVP$. On the other hand, DDAVP, a V_2 receptor agonist, evoked only moderate antipyresis compared to AVP and thus, DDAVP action in this case was attributed to interaction of this agonist with V_{1a} receptors.

The role of endogenously released AVP in fever suppression was investigated, as it relates to the meA. Bilateral injection of the V_{1a} antagonist, $d(CH_2)_5$Tyr(Me)AVP, into the meA augmented PGE_1-evoked hyperthermia during the latter stages of defervescence. In contrast, similar injection of the V_2 antagonist, $d(CH_2)_5$-D-ValVAVP, did not alter the time course of PGE_1 hyperthermia. This suggests that endogenously released AVP might act within the meA on a V_{1a}-like receptor to limit the duration of PGE_1 hyperthermia during defervescence.

In conclusion, the findings reported in this thesis support and strengthen the hypothesis that vasopressin might function within the meA as part of an endogenous antipyretic system.

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DEDICATION

To my parents and Tanya for their love, continuing support and patience.

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LIST OF ABBREVIATIONS

aCSF	Artificial cerebrospinal fluid
AVP	Arginine vasopressin
BP	Bacterial pyrogen
BSA	Bovine Serum Albumin
BST	Bed nucleus of the stria terminalis
°C	Degrees centigrade
CNS	Central nervous system
CSF	Cerebrospinal fluid
DDAVP	Desamino-D-arginine-8-vasopressin
EEG	Electroencephalogram
EP	Endogenous pyrogen
g	gram
ga	Gauge
h	Hour
icv	Intracerebroventricular
IL-1	Interleukin 1
ip	Intraperitoneal
iv	Intravenous
kg	Kilogram
LCV	Lateral cerebral ventricle
M	Molar

meA	Medial amygdaloid nucleus
mg	Milligram
min	Minute
ml	Millilitre
mm	Millimetre
α-MSH	Alpha-melanocyte stimulating hormone
ng	Nanogram
OVLT	Organum vasculosum lamina terminalis
PBS	Phosphate buffer saline
PGE	Prostaglandin
PGE_1	Prostaglandin E_1
PGE_2	Prostaglandin E_2
pmol	Picomole
POAH	Preoptic/anterior hypothalamic area
PVN	Paraventricular nucleus
s	Second
SCN	Suprachiasmatic nucleus
SEM	Standard error of the mean
U	Units of biological activity
VSA	Ventral septal area
YSI	Yellow springs instruments
μg	Microgram
μl	Microlitre
μM	Micromolar

CHAPTER 1

INTRODUCTION

A. THERMOREGULATION AND THE CENTRAL NERVOUS SYSTEM

The regulation of body temperature within strict physiological limits is a homeostatic process essential to the survival of mammals and other species. Indeed, in times of physiological stress, temperature regulation takes precedence over other regulated systems. This fundamental relationship between heat and life has long been recognized by the ancient greeks including Hippocrates and Galen (Sigal, 1978; Kluger, 1980). It was not until the early part of the 19th century, however, that the central nervous system (CNS) was correctly implicated in regulating body temperature by balancing heat loss and heat gain mechanisms (Currie, 1798; Wunderlich, 1871; Chossat, 1820).

Studies elucidating the central location of temperature regulating centres revealed that the hypothalamus is of principal importance. Specifically, Isenschmid and Schnitzler (1914) demonstrated that massive lesions of the hypothalamus abolished thermoregulation. Other studies utilizing more discrete lesioning techniques revealed that lesions of the posterior and anterior hypothalamus cause the respective loss of heat production and heat dissipation mechanisms (Clark et al., 1939; Andersson et al., 1965; Squires and Jacobson, 1968; Carlisle, 1969; Satinoff and Rustein, 1970). In addition, lesions of the lateral hypothalamus selectively impair behavioural thermoregulatory responses while autonomic thermoregulatory mechanisms are ameliorated by lesions of the preoptic/anterior hypothalamus

(POAH; Satinoff and Shan, 1971; Satinoff and Rustein, 1970). Furthermore, it was found that both local temperature changes (Beaton et al., 1941; Calvert and Findlay, 1975) and electrical stimulation (Andersson et al., 1956; Andersson, 1957; Hemingway et al., 1954) of the POAH produces thermoregulatory changes. Thus, the hypothalamic region appears to be a brain locus important in thermoregulatory processes.

B. FEVER

1) Introduction

Fever is a pathological condition in which there is a regulated elevation in body temperature up to 5°C above normal. Febrile rises in core temperature are actively defended against hot or cold thermal challenges (Cooper et al., 1964; MacPherson, 1959) and thus, fever is distinct from hyperthermia which is a nonregulated passive increase in body temperature (Stitt, 1979).

In 1871, Leibermaster postulated that the CNS is important in the generation and maintenance of febrile responses. Support for this comes from studies demonstrating that lesions of the posterior hypothalamus reduce febrile responses to pyrogens (Thompson et al., 1959; Cooper and Veale, 1974) and studies showing that pyrogens probably exert their effects in the anterior hypothalamus (Bennet et al., 1957; Villablanca and Myers, 1965; Cooper et al., 1967; Jackson, 1967).

2) Central Mechanisms of Fever Generation

a) Set Point Theory

The central mechanisms employed to generate and maintain febrile body

temperatures are poorly understood. One central mechanism has been proposed in which body temperature is actively regulated around an arbitrary "set point" in a manner analogous to a thermostat (Leibermaster, 1871; Barbour, 1921). In fever, this set point is raised to a higher level and as a result, body temperature is actively raised to this new level by increasing heat production and/or decreasing heat dissipation. Supporting this theory are studies demonstrating that the body actively defends febrile body temperature against hot or cold thermoregulatory challenges (MacPherson, 1959; Myers and Veale, 1971; Cooper et al., 1964).

Myers and Veale (1971, 1972) described a potential mechanism for set point alteration during fever. Specifically, they proposed that the set point about which body temperature is regulated depends on the ratio of [Na^+] to [Ca^{++}] in the extracellular fluid of the posterior hypothalamus. This suggestion was based on the observation that infusion of excess [Ca^{++}] into the posterior hypothalamus causes animals to regulate at a lower body temperature while excess [Na^+] in the same site caused animals to regulate at febrile temperatures. Further support of this ionic mechanism comes from the observation that radioactively labelled endogenous stores of [Na^+] and [Ca^{++}] change accordingly during the development of fever and during defervescence (Myers and Tyrell, 1972).

b) <u>An Alternative to the Set Point Theory</u>

Numerous studies have demonstrated that the activity and thermosensitivity of single units in the anterior hypothalamus are altered by pyrogen administration in a manner anticipated for increased heat storage that would lead to febrile increases in body temperature (Cabanac et al., 1968; Eisenman, 1969; Eisenman,

1974; Wit and Wang, 1968; Eisenman, 1982). It was therefore suggested that pyrogens might induce fever by altering neuronal activity in key thermoregulatory centres rather than displacing thermoregulatory set point. Unfortunately, the physiological significance of this altered neuronal activity has proven difficult to assess.

3) Pyrogens

a) Exogenous Pyrogen

Fever can be initiated by a wide range of agents including endotoxins, viruses, and gram-positive bacteria. These agents are termed exogenous pyrogens or bacterial pyrogens (BP) since they exist outside the body (ie. exogenous) and can induce fever (ie. pyrogenic). The most potent exogenous pyrogens are endotoxins which are derived from the cell wall of gram-negative bacteria such as *Escherichia*, *Salmonella*, and *Shigella*. These exogenous pyrogens are large, 1-2 million molecular weight molecules with three identifiable subunits; an O-specific polysaccharide, a core polysaccharide and a lipid A fraction (Luderitz et al., 1971; Work, 1971). It is believed that the lipid A fraction is what bestows most of the toxic and biologic activities to endotoxin (Galanos et al., 1972; Reitschel et al., 1973).

Due to their large molecular weight, exogenous pyrogens are believed to be unable to pass from the peripheral circulation, across the blood brain barrier, into the CNS (Rowley et al., 1956; Braude et al., 1955; Cooper and Cranston, 1963; Dascombe and Milton, 1979; Trippodo et al., 1973). Thus, if the CNS were to be involved in evoking fevers, some additional substance, or mediator, is required.

b) Endogenous Pyrogen

A common process is believed to be responsible for the genesis of fever by exogenous pyrogen that involves the activation of the reticuloendothelial system. Specifically, various phagocytic cells of the reticuloendothelial system destroy and engulf exogenous pyrogens and simultaneously release a pyrogenic material initially termed endogenous pyrogen (EP), but now known as interleukin-1 (IL-1; Dinarello, 1984). Interleukin-1 has proven difficult to purify and because of certain heterogeneity, IL-1 appears to exist as a family of polypeptides. Indeed, IL-1 demonstrates charge heterogeneity since it is characterized by 3 or more isoelectric points, thus indicating the presence of 3 or more closely related compounds of differing weights (Wood et al., 1985). With the recent advent of recombinant DNA technology, it has been possible to demonstrate that there exist at least 2 distinct forms of IL-1 derived from different gene products (Auron et al., 1985). Such recombinant DNA technology was also instrumental in showing that different cell types in the same animal produce different forms of interleukins which elicit unique febrile responses (Dinarello, 1984; LeMay et al., 1990).

The cells important in IL-1 production include blood monocytes, fixed macrophages in the blood, liver, and spleen as well as tissue macrophages in body cavities, joints, bone marrow and lymph nodes (Beeson, 1948; Bodel and Atkins, 1967; Atkins et al., 1967; Dinarello et al., 1968; Dinarello, 1984). In addition, glial cells have been found to be capable of producing IL-1 (Fontana et al., 1982; Fontana et al., 1984a) and such central IL-1 synthesis has been observed in mice following peripheral pretreatment with endotoxin (Fontana et al., 1984b). Thus, a wide variety

of cells, both central and peripheral, are capable of Il-1 synthesis. Little is known, however, about the precise mechanism(s) whereby exogenous pyrogens stimulate IL-1 synthesis and release.

4) Central Mediators of Fever

An interaction between circulating IL-1 and the CNS is responsible for the thermal component of fever. Since it is possible that IL-1 cannot enter the CNS (see later), there has been a search for central mediators of fever which might be involved in raising core temperature during fever.

a) Protein Mediators

Evidence exists suggesting that a mechanism by which IL-1 elicits fever is through protein synthesis. Specifically, inhibition of protein synthesis by systemic treatment with cyclohexamide was found to attenuate fevers produced by endotoxin (Siegert et al., 1976) and crude IL-1 (Siegert et al., 1976; Cranston et al., 1978). However, the relevance of these experiments have been questioned since cyclohexamide was later found to have additional nonspecific effects such as incapacitating the ability to thermoregulate (Barney et al., 1979; Stitt, 1980).

Anisomycin is another protein synthesis inhibitor that, unlike cyclohexamide, does not compromise the thermoregulatory capacity of animals (Cranston et al., 1980; Ruwe and Myers, 1980). Systemically administered anisomycin was found to attenuate fevers induced by either central or peripheral pyrogen administration (Ruwe and Myers, 1979; Ruwe and Myers, 1980; Cranston et al., 1980; Cranston et al., 1982). Furthermore, central injection of anisomycin into the POAH delays or

prevents endotoxin fevers, suggesting that the POAH may be an important site where de novo protein synthesis occurs in the development of fever (Ruwe and Myers, 1980).

Despite compelling evidence implicating an unknown protein mediator in the genesis of fever, the mechanism whereby protein synthesis contributes to the development of febrile rises in core temperature is not known. Currently, as reviewed by Milton and Sawheny (1980), much of the work related to this subject is confusing and conflicting. Thus, further work is necessary before any role can be ascribed to hypothalamic protein synthesis in fever.

b) Prostaglandins

Prostaglandins of the E series (PGE) have been suggested to play an important neurotransmitter or neuromodulatory role in the genesis of febrile body temperatures based on several findings. First, PGE evokes a dose-related hyperthermia when injected into the cerebral ventricles or into the POAH of numerous species including cats, rabbits, mice, guinea-pigs, chickens, and monkeys (Milton and Wendlandt, 1971; Feldberg and Saxena, 1971; Hales et al., 1973; Crawshaw and Stitt, 1975; Veale and Whishaw, 1976; Coceani et al., 1983; Bernheim et al., 1980; Milton, 1982). Second, prostaglandin levels in the CSF increase during the development of fever and decrease during defervescence (Feldberg and Gupta, 1973; Phillip-Dormstrom and Siegert, 1974; Bernheim et al., 1980; Coceani et al., 1983). Third, antipyretic drugs have been shown to inhibit prostaglandin synthesis (Flower and Vane, 1972; Vane, 1971) and to decrease CSF levels of PGE in parallel with body temperature in febrile animals (Feldberg and Gupta, 1973; Feldberg et

al., 1973). Fourth, electrophysiological studies have demonstrated that intracerebroventricular (i.c.v.) injection or direct administration of PGE into the POAH alters the firing rate and intrinsic electrophysiological properties of neurons in this site (Ford, 1974; Gordon and Heath, 1979).

Evidence has also been adduced refuting the role of PGE as a mediator of febrile responses. For example, Cranston et al. (1975) demonstrated that the increased CSF levels of PGE_2 observed during fever could be prevented by salicylate adminstration in sufficiently low doses that do not affect febrile responses. In addition, i.c.v. injections of 2 separate prostaglandin antagonists (SC 19220 and Hr 546) blocked PGE-induced hyperthermia but not fevers produced by i.c.v. administration of IL-1 or sodium arachidonate (Cranston et al., 1976; Laburn et al., 1977), thus indicating that PGE elaboration and action is not essential for the genesis of fever. Furthermore, bilateral lesions of the POAH in rabbits resulted in the abolishment of PGE hyperthermia while IL-1 fevers were largely unaltered (Veale and Cooper, 1975; Cooper et al., 1976). Some electrophysiological studies also refute a role for PGE as a mediator of fever since in these studies, only 10% of POAH neurons responded to direct application of PGE and not in a consistent manner (Jell and Sweatman, 1977; Stitt and Hardy, 1975; Krettek and Price, 1977). These findings therefore suggest that PGE may not be a crucial mediator of the febrile response.

In summary, it appears that centrally synthesized and acting PGE might be involved in some capacity as a central mediator of pyrogen fever, though it may not be an absolute requisite.

c) Others

Several putative neurotransmitters have been identified as possible central mediators of fever. These include acetylcholine, 5-hydroxytryptamine, noradrenaline, as well as various peptides and cyclic nucleotides. Since most data regarding these interactions are confusing and oftentimes conflicting, no discussion of this topic will be carried out. For a detailed review of this topic see Dascombe (1985).

C. MECHANISM OF ACTION OF ENDOGENOUS PYROGEN

It is generally accepted that exogenous pyrogens stimulate the synthesis and release of endogenous pyrogen (IL-1) as part of the first step in the genesis of fevers. What occurs following this step, though widely studied, is still debated. The following sections will review some findings on the mechanism of IL-1 action.

1) Site of Action

a) Organum Vasculosum Lamina Terminalis

It is believed that IL-1, either elaborated within the circulation in response to exogenous pyrogen, or systemically administered, cannot cross the blood brain barrier to enter the brain neuropil (Dinarello et al., 1978; Blatteis et al., 1989). Furthermore, i.c.v. administered crude IL-1 evokes fevers with onset latencies and overall durations of more than twice that of fevers elicited by i.v. IL-1. This finding indicates that the site of IL-1 action may be closer to the peripheral circulation than the cerebrospinal fluid (Stitt and Bernheim, 1985). To explain these findings, Stitt (1985, 1986) proposed that the organum vasculosum lamina terminalis (OVLT) may

be involved in the mediation of the febrile event. The OVLT is a circumventricular organ located in the rostral wall of the third ventricle adjacent to the POAH but outside the blood brain barrier (Weindl, 1969). This location, along with the absence of tight junctional vascular endothelium, allows larger molecules to interact within the perivascular space of the OVLT hence making this structure a potential site of IL-1 action.

Blatteis et al. (1983) provided the first evidence implicating the OVLT in the genesis of fever when it was demonstrated that lesions of the anteroventral portion of the third ventricle abolished endotoxin fevers in guinea-pigs. Later, in apparent contradiction, Stitt (1985) showed that discrete lesions within the confines of the OVLT augment IL-1 fevers in rats and rabbits. This discrepancy between the two studies was attributed to the extent of OVLT lesioning. Specifically, Blatteis et al. (1983) may have functionally eliminated the OVLT whereas this structure remained intact in the study of Stitt (1985). Indeed, it was postulated that the enhanced sensitivity to pyrogens following partial lesioning of the OVLT was due to the sensitization of a reticuloendothelial cell within this site, perhaps as part of an inflammatory or irritative response (Stitt, 1985; 1986). Support of this possibility comes from studies in which the immuno-adjuvant zymosan, microinjected into the OVLT, resulted in enhanced fevers that were similar to those elicited following discrete lesioning of the OVLT (Stitt et al., 1984; Stitt and Shimada, 1989). Also consistent with this possibility is the presence of mesenchymally-derived phagocyte cells within the OVLT (Murabe et al., 1981). According to Stitt (1986), these cells might possess specific receptors for IL-1, thus making them the target of IL-1 action. However, whether IL-1 receptors are actually present on any cell type within the

OVLT remains to be determined.

In summary, it is possible that IL-1 might act within the OVLT via an IL-1 receptor located on a phagocytic cell to evoke febrile rises in body temperature.

b) Preoptic/Anterior Hypothalamic Area

The POAH may be another site where IL-1 ultimately exerts its pyrogenic effects. This contention is based on the observation that microinjection of IL-1 into the POAH, but not surrounding areas, evokes fevers with a characteristic latency and duration (Cooper et al., 1967; Jackson, 1967). Electrophysiological studies also revealed that both systemically administered bacterial pyrogen or crude IL-1 (Wit and Wang, 1968; Cabanac et al., 1968; Eisenman, 1969; Eisenman, 1974) as well as IL-1 microinjected directly into the POAH (Wit and Wang, 1968; Schoener and Wang, 1975) results in a depression of activity and thermosensitivity of warm sensitive neurons and an enhancement of activity and thermosensitivity cold sensitive neurons in the POAH. Studies using the *in vitro* hypothalamic slice also demonstrated similar IL-1 actions (Hori et al., 1984). In addition, IL-1 effects on POAH neurons are reversed by antipyretics (Wit and Wang, 1968; Schoener and Wang, 1975) and appear to be those appropriate for the decrease in heat loss and increase in heat production observed during the rising phase of fever (Eisenman, 1982). However, the physiological significance of the depressed thermosensitivity in POAH neurons is unclear since no decrease in thermoregulatory ability is observed during fever. It has been shown, however, that following pyrogen administration, animals are less sensitive to POAH thermal stimulation (Eisenman, 1974; Lipton and Kennedy, 1979).

In summary, evidence suggests that IL-1 might act within the POAH to elicit fevers by altering neuronal thermosensitivity in this region.

c) Central IL-1 Synthesis

There is concern that central injection of IL-1 into neural tissue or into the ventricular system (as described in the previous section) may represent an artificial situation since it is possible that IL-1 cannot enter the CNS (Dinarello et al., 1978) and since the characteristics of i.v.versus i.c.v. IL-1 fevers differ (Stitt and Bernheim, 1985). However, if cells within the brain were to synthesize and release IL-1, then the physiological relevance of such studies would not be called into question. Supporting this possibility, astrocytes and glioma cells have been shown to be capable of IL-1 synthesis (Fontana et al., 1982; Fontana et al., 1984a; Dinarello and Bernheim, 1981). In addition, the induction of IL-1 synthesis has been demonstrated in mice brains following systemic administration of pyrogen (Fontana et al., 1984b). However, the mechanism whereby peripheral pyrogen is linked to central IL-1 synthesis is unknown.

2) Mechanism of Action

a) Central Prostaglandin Synthesis

Earlier, the possibility that IL-1 actions may involve intermediaries such as the prostaglandins was reviewed. To further examine this possibility, evidence concerning IL-1-induced PGE synthesis as well as PGE sites of action will be reviewed.

i) Preoptic/Anterior Hypothalamic Area

Relatively few studies have identified central sites releasing PGE during fever since most studies focused on correlating CSF levels of PGE with body temperature. Despite this, a few *in vitro* studies have shown that IL-1 can induce PGE synthesis in the anterior hypothalamic slice (Dinarello and Bernheim, 1981; Fontana et al., 1982), thus supporting the contention that IL-1 might act at this site in the normal genesis of fever.

Evidence has been adduced supporting a role for PGE acting within the POAH in the genesis of fevers (see section B-4b). Briefly, PGE microinjected into POAH of numerous species, evokes prompt febrile increases in body temperature (Eisenman, 1982). In contrast, PGE injections in the posterior hypothalamus or midbrain reticular formation did not evoke any consistent effects on body temperature. In an extensive exploration for PGE sensitive sites in the brain, Williams et al. (1977) found that PGE sensitive sites were primarily localized to the POAH.

Electrophysiological studies also confirm a role for PGE in the POAH in the genesis of fever. Specifically, i.c.v. injection or direct administration of PGE onto neurons in this site inhibited warm sensitive neurons and facilitated cold sensitive neurons (Ford, 1974; Gordon and Heath, 1979), changes that could be reversed by local application of salicylate (Hori et al., 1984; Wit and Wang, 1968).

ii) Organum Vasculosum Laminae Terminalis

Recently, Stitt (1986; 1990) demonstrated that the OVLT is sensitive to the hyperthermic effects of exogenously administered PGE and that this sensitivity

greatly exceeds that of PGE injected into the cerebral ventricles or POAH. Based on this finding, it has been postulated that IL-1 might act within this site on a target cell (see earlier) to evoke the release of PGE. Following this, two possible courses of PGE action were proposed. The first is that PGE, which is a small lipophilic molecule, crosses the blood barrier separating the OVLT from the POAH and enters the neuropil of the POAH to evoke fevers by altering neuronal thermosensitivity at this site (Stitt, 1986). A second, more speculative possibility, is that PGE might act within the OVLT (rather than the POAH) on neuronal elements that project to the POAH (Stitt, 1986). This last possibility was suggested as an explanation of the high PGE sensitivity of the OVLT compared to the POAH.

b) Role of Ca^{++} Channels

Dinarello (1984) has proposed that the cellular actions of IL-1 are probably based on increasing intracellular calcium in the target cell. This in turn, would activate several second messenger systems. Support for this action comes from the observation that biological actions characteristic of IL-1 are mimicked by the calcium ionophore A23187. Some of these actions include increasing PGE synthesis by a calcium-dependant activation of phoshpolipase A_2, an enzyme necessary for PGE synthesis (Betteridge, 1980; Stitt, 1986), mitogenesis of T cells (Luckasen et al., 1974) and neutrophil degranulation (Klempner et al., 1978).

Given that IL-1 might stimulate PGE synthesis by a calcium-dependant activation of phoshpolipase A_2, the role of calcium channel activation in the genesis of fever has been investigated. Stitt and Shimada (1985) found that the calcium channel blocker, vermapil, administered i.v. or directly into the OVLT attenuates IL-

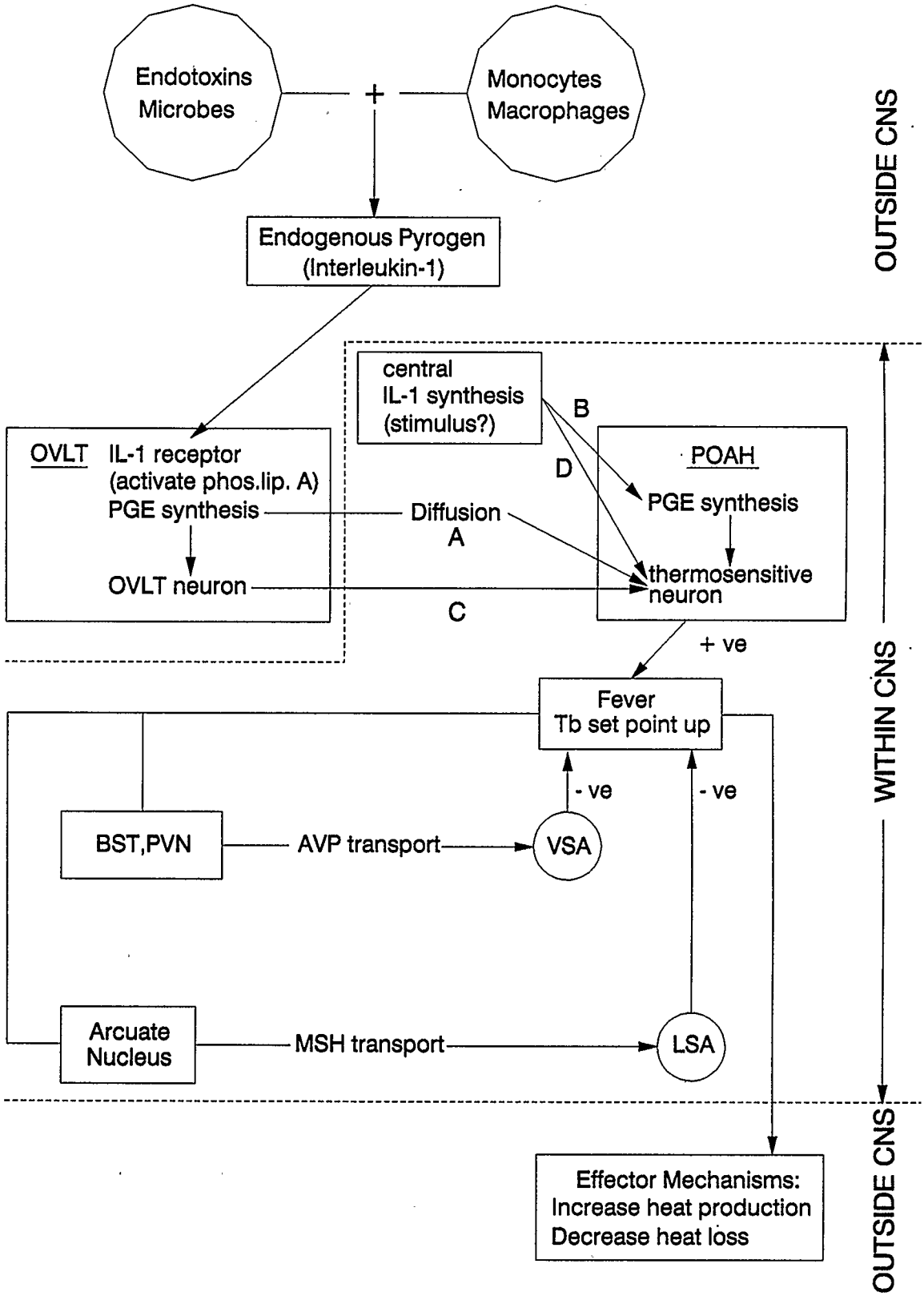
1 (injected i.v.) but not PGE (injected into the OVLT) induced fevers. Thus, it was suggested that the mechanism of action of IL-1 involves calcium channel activation. This finding also strengthened the hypothesis that the OVLT may be an important site involved in the transduction of the IL-1 signal into the brain. These data are also consistent with the possibility that IL-1 might stimulate PGE synthesis within the OVLT by a calcium dependant activation of phosopholipase A_2. However, this latter suggestion must be viewed with caution since the blockade of calcium channels can have many intracellular effects in addition to inhibiting PGE synthesis.

3) Summary

Summarized in Fig.1 are proposed mechanisms of fever and antipyresis (see later) based on current experimental evidence. In addition, possible mechanisms of IL-1 action are summarized (Fig. 1, A-D). Note that any single mechanism or combination of mechanisms of IL-1 action might be involved in the genesis of fever.

It is possible that the IL-1 signal is transducted into the brain via the OVLT. This transduction event might involve a receptor on a target cell, calcium channels, activation of phosholipase A_2 and finally, PGE synthesis within the OVLT. In addition, centrally released IL-1 might act within the POAH to stimulate PGE synthesis and release. Prostaglandin E synthesized within the OVLT and POAH may, in turn, act within the POAH to evoke febrile rises in body temperature by altering neuronal thermosensitivity (Fig 1, A and B). In the genesis of fevers, PGE synthesized in the OVLT has also been suggested to act on OVLT neurons which may, in turn, modulate POAH thermosensitive neurons (Fig 1, C). In addition, PGE synthesized within the POAH might diffuse to the OVLT to act on neuronal

Fig. 1. Schematic diagram illustrating the sequence of steps in the genesis of fever as well as components of 2 endogenous antipyretic systems, based on current experimental evidence. Abbreviations: AVP, arginine vasopressin; AH/POA, anterior hypothalamic/preoptic area; BST, bed nucleus of the stria terminalis; CNS, central nervous system; IL-1, interleukin-1; LSA, lateral septal area; MSH, melanocyte stimulating hormone; OVLT, organum vasculosum of the lamina terminalis; PGE, prostaglandin E; PVN, paraventricular nucleus of the hypothalamus; Tb, body temperature; VSA, ventral septal area.


Endotoxins
Microbes
+
Monocytes
Macrophages
Endogenous Pyrogen
(Interleukin-1)
OUTSIDE CNS
central
IL-1 synthesis
(stimulus?)
B
D
OVLT
IL-1 receptor
(activate phos.lip. A)
PGE synthesis
Diffusion
A
OVLT neuron
C
POAH
PGE synthesis
thermosensitive
neuron
+ ve
Fever
Tb set point up
WITHIN CNS
- ve
- ve
BST,PVN
AVP transport
VSA
Arcuate
Nucleus
MSH transport
LSA
Effector Mechanisms:
Increase heat production
Decrease heat loss
OUTSIDE CNS

elements there (not shown in Fig. 1). Finally, IL-1 might directly act on POAH neurons to elicit febrile rises in body temperature (Fig 1, D).

D. ANTIPYRETICS

Antipyretic substances, as defined by Barbour (1921), are substances that reduce body temperature in febrile and similar pathological conditions, but not in normal conditions unless the dosage is excessive. Antipyretic drugs can be steroidal or non-steroidal in nature though the majority of study on antipyretics has been directed at non-steroidal antipyretic drugs including aspirin (sodium salicylate), acetaminophen, and indomethacin. In addition to classical antipyretic drugs such as the salicylates, endogenous neuropeptides including arginine vasopressin (Cooper et al., 1979) and melanocyte stimulating hormone (Glyn and Lipton, 1981) have been identified as being antipyretic.

1) Classical Antipyretics

a) Site of Action

It is possible that classical antipyretic drugs may exert their effects by interfering with fever development at any point along the febrile pathway. However, it has been shown that classical antipyretics do not interfere with IL-1 production or release (Van Miert et al., 1972; Lin and Chai, 1972), nor do they inactivate the molecule (Hoo et al., 1972) or prevent its entry into the CNS (Cranston et al., 1970; Lin and Chai, 1972; Clark and Cumby, 1975; Chai et al., 1971). Antipyretic drugs were consequently proposed to act within the CNS, specifically within the POAH, where pyrogens probably exert their pyretic activity. In support of this, antipyretics

microinjected into the POAH, effectively attenuate fevers elicited by peripheral administration of endotoxin and IL-1 (Lin and Chai, 1972). Furthermore, salicylate, microinjected into the POAH, was found to inhibit the pyrogenic effects of crude IL-1 injected into the same site (Schoener and Wang, 1975). Thus, it appears that antipyretics might act within the POAH to reduce fevers. However, the possibility that antipyretics might act within the OVLT to reduce fevers has yet to be investigated.

b) Mechanism of Action

It has been suggested that antipyretics may exert their effects by inhibiting IL-1-induced prostaglandin synthesis since early studies have shown that antipyretics inhibit prostaglandin synthesis (Vane, 1971; Flower and Vane, 1972). In support of this, a significant correlation exists between antipyretic potencies and prostaglandin synthesis inhibition in the rat (Ziel and Krupp, 1975). Further, exogenous administration of antipyretic substances in febrile animals evokes parallel decreases in both body temperature and CSF levels of PGE (Feldberg et al., 1973). Lastly, antipyretics injected i.c.v. do not alter the hyperthermic effects of PGE given i.c.v. (Milton, 1982; Woolf et al., 1975), indicating that antipyretics can not alter the hyperthermic effects of PGE, once synthesized.

In contrast to the last finding, salicylate and indomethacin infused into a diencephalic brain site, the ventral septal area, was found to suppress hyperthermia evoked by the i.c.v. administration of PGE (Fyda et al., 1990; Alexander et al., 1987; Fyda et al., 1989). Thus, there may be an additional mechanism of action of antipyretics other than the inhibition of PGE synthesis. Regarding this, salicylate

and indomethacin have been proposed to act within the ventral septal area by stimulating the release of vasopressin, a proposed endogenous antipyretic substance (Alexander et al., 1987; Fyda et al., 1989; Fyda et al., 1990; see later). However, the mechanism whereby these classical antipyretic drugs evoke the release vasopressin is not known.

Since it is known that pyrogens can alter the activity of neurons in the POAH, antipyretics may also act by antagonising IL-1 or PGE effects at this site. Support for this stems from the observation that salicylate reverses pyrogen-induced changes in neuronal activity and thermosensitivity in the POAH (Wit and Wang, 1968; Schoener and Wang, 1975; Hori et al., 1984).

In summary, it appears that classical antipyretics might act within the POAH to attenuate fevers by inhibiting PGE synthesis or by antagonizing pyrogen-induced changes in POAH neuronal activity and thermosensitivity. Furthermore, classical antipyretics might act to evoke antipyresis in another brain site, the ventral septal area, by eliciting the release of vasopressin within this site through an unknown mechanism.

2) Endogenous Antipyresis and Vasopressin

It has long been recognized that fevers rarely exceed 4°C above normal body temperature (Dubois, 1949). This suggests that the body possesses some form of negative feedback which prevents the magnitude of febrile responses from reaching harmfully excessive levels. The next sections will review the concept of endogenous antipyresis and the role that the neuropeptide arginine vasopressin (AVP) may play in this process.

a) Fever at Term

Numerous studies have reported that the febrile response to either endotoxin or crude IL-1 is reduced in the pregnant Suffold cross ewe (Kasting et al., 1978) and guinea-pig (Zeisberger et al., 1981) from 4-5 days prepartum to 5 hours after delivery. It was also found that the reticuloendothelial system of these animals retained the ability to synthesize and release IL-1 (Kasting et al., 1979b). Thus, it was reasoned that the refractoriness to pyrogens at term was not due to the inability of these animals to generate fevers, but instead resulted from increased levels of an endogenous antipyretic substance.

Of the many hormonal fluctuations that occur during pregnancy, plasma levels of arginine vasopressin were found to correlate well with the occurrence of pyrogen tolerance (Alexander et al., 1974). Consistent with this finding, anatomical studies demonstrated the activation of central vasopressinergic neurons in the pregnant guinea-pig at term (Merker et al., 1980; Zeisberger et al., 1981). Specifically, AVP levels (determined immunocytochemically) increase in cell bodies in the paraventricular nucleus and in nerve terminals within the septum and amygdala of these animals. Thus, it is possible that the refractoriness to pyrogen of animals at term may be due to increased central levels of vasopressin.

b) The Ventral Septal Area and Endogenous Antipyresis

i) Site of Action

A functional role for AVP in endogenous antipyresis was demonstrated for the first time in sheep by Kasting et al. (1979a). Specifically, when AVP was perfused within the ventral septal area (VSA) of the sheep brain, fevers evoked by

systemic endotoxin were suppressed in a concentration dependant manner. The site of AVP antipyretic action, namely the VSA, is located ventral to the lateral septum and lateral to the vertical limb of the diagonal band of Broca. Cooper et al. (1979) provided further evidence that the antipyretic action of AVP is centrally mediated by demonstrating that systemic administration of the peptide does not result in any fever suppression. Consistent with the requirement of a true antipyretic, AVP exogenously administered within the VSA, does not alter afebrile body temperature (Kasting et al., 1979a) unless the dosage is excessive (Kasting et al., 1980; Kruk and Brittain, 1972; Meisenberg and Simmons, 1984).

The brain site where exogenously administered AVP suppresses fever is similarly located in the VSA in other species including the rat (Ruwe et al., 1985), cat (Ruwe et al., 1985b; Ruwe et al., 1986), rabbit (Naylor et al., 1985b) and guinea-pig (Zeisberger, 1989; Naylor, 1987). Further, AVP is not antipyretic when administered outside the VSA into the lateral, anterior or posterior hypothalamus, the preoptic area, the fornix, or the dorsal septum (Cooper et al., 1979; Bernardini et al., 1983).

ii) Experimental Evidence

The central release of AVP into AVP sensitive antipyretic sites has been used as evidence that the peptide functions under physiological conditions as an endogenous antipyretic. The first such evidence was provided by Cooper et al. (1979) when they showed that during fever, AVP levels in push-pull perfusates taken from the VSA of the sheep brain correlated negatively with changes in body temperature. Thus, as body temperature decreased during defervescence, AVP

levels in the extracellular fluid of the VSA increased. Similarly, AVP levels in septal perfusates decreased as body temperature rose during fever. This negative correlation between AVP release into the VSA and febrile body temperature has also been reported for the rabbit (Ruwe et al., 1985b).

Further evidence for the release of AVP during fever comes from anatomical studies where in response to systemic endotoxin, AVP concentrations (determined by radioimmunoassay) in the septum, amygdala and caudate nucleus decrease, while AVP levels remained unchanged in other areas (Kasting and Martin, 1983). This decrease in immunoreactive AVP levels was interpreted as an increase in AVP release. In addition, immunocytochemical investigations report changes in immunoreactive AVP levels in nerve terminals of the septum and amygdala as well as in paraventricular soma in febrile guinea-pigs (Zeisberger et al., 1983; Cooper et al., 1988; Zeisberger et al., 1986). These changes were also taken as evidence supporting the central release of AVP during fever.

Studies augmenting central AVP release support a role for the peptide in endogenous antipyresis. For example, augmenting central AVP release by haemorrhage or peripheral hypertonic saline (Kasting, 1986) results in a suppression of fevers. Furthermore, electrical stimulation of the principle source of AVP to the VSA, the bed nucleus of the stria terminalis (BST), suppresses PGE_1-evoked hyperthermia, an action that could be reversed with AVP antagonists (Naylor et al., 1988). In addition, the activity putative vasopressinergic neurons in the BST has been shown to increase during defervescence in the rat (Mathieson et al., 1989a). Thus, during fever, it is possible that vasopressinergic neurons in the BST are activated (by an unknown stimulus), thereby releasing AVP into the VSA where this

peptide then exerts its antipyretic effects.

Studies blocking AVP action also implicate a role for AVP as an endogenous antipyretic acting within the VSA. Exogenous administration of specific AVP antisera (Malkinson et al., 1987; Kasting, 1980) or AVP antagonists (Cooper et al., 1987; Naylor et al., 1988; Landgraf et al., 1990) in the VSA results in enhanced fevers, presumably by preventing endogenously released AVP from modulating fever through its interaction with receptors in the VSA.

Electrophysiological studies have documented the presence of thermoresponsive neurons in the VSA which therefore implicates this site as a component of thermoregulatory pathways in the brain (Disturnal et al., 1986). Furthermore, the observation that the majority of these neurons were cold responsive indicates that the VSA might be involved in regulating heat loss. Evidence that AVP might be associated with antipyresis in this site was provided by the finding that the activity of thermoresponsive neurons in this area was modified by stimulating putative vasopressinergic projections arising from the BST and paraventricular nucleus (Disturnal et al., 1985). Furthermore, it has been shown that iontophoretic application of AVP onto VSA neurons inhibit the excitatory effects of glutamate at this site and that this inhibition can be reversed by AVP antagonists (Disturnal et al., 1987).

In summary, it appears from a wide variety of evidence that vasopressin may act as an endogenous antipyretic within the VSA. The mechanism by which AVP acts within the VSA to modulate fever is not known though it could involve an alteration of the thermoregulatory set point (Naylor, 1987; Wilkinson and Kasting, 1986) via neuroanatomical connections with thermoregulatory centres in the

hypothalamus and preoptic area (Garris, 1979; Naylor, 1987).

c) Convulsive Disorders and Vasopressin

It has been demonstrated that i.c.v. injection of vasopressin evokes, via a sensitisation process, convulsive behaviour characterized by myotonic/myoclonic seizures and barrel rotations (Kruse et al., 1977; Kasting et al., 1980). Further, the brain site mediating this action of AVP was found to be the VSA, where the peptide is also antipyretic (Naylor et al., 1985). The occurrence of AVP release into the VSA during fever (Cooper et al., 1979), along with the convulsive action of the peptide in this site, raises the possibility that AVP might be involved in the development of febrile convulsions. Consistent with this possibility is the finding that Brattleboro rats, which lack endogenous vasopressin (Valtin et al., 1965) demonstrate an increased threshold for hyperthermic convulsions compared to control Long Evans rats (Kasting et al., 1981). Further, a vasopressin antagonist that blocked the antipyretic effect of AVP (Kasting and Wilkinson, 1986) also blocked its convulsive effects (Naylor et al., 1985; Burnard et al., 1986). Thus, AVP released into the VSA during fever may be involved in the etiology of febrile convulsions.

3) Endogenous Antipyresis and Melanocyte Stimulating Hormone

Evidence has been adduced suggesting that melanocyte stimulating hormone (α-MSH), a peptide derived from pro-opiomelanocortin (O'Donohue and Dorsa, 1982), may play a role in fever suppression since α-MSH can reduce pyrogen fevers in the rabbit (Glyn and Lipton, 1981; Murphy et al., 1983), guinea-pig (Kamdasamy and Williams, 1984), squirrel monkey (Shih and Lipton, 1985) but not the cat (Rezvani et al., 1986).

The neuroanatomical locus mediating α-MSH action appears to be the lateral septum since microinjection of the peptide into this site, but not surrounding regions, resulted in fever suppression (Glyn-Ballinger et al., 1983). Consistent with this finding are anatomical studies describing melanotropic soma in the arcuate nucleus of the hypothalamus that project to extrahypothalamic sites including the lateral septum (Eskay et al., 1979; O'Donohue and Dorsa, 1982).

Evidence for the endogenous release of α-MSH into the lateral septum during fever comes from studies demonstrating that the increase in body temperature during the rising (chill) phase of fever is associated with an increase in immunoreactive α-MSH levels in push-pull perfusates taken from this site (Bell and Lipton, 1987). This release was also found to be specific to fever and not to hyperthermia (Samson et al., 1981; Holdeman et al., 1985). Furthermore, sequestering central α-MSH with i.c.v. injections of α-MSH antiserum (Shih et al., 1986) or depleting central α-MSH by lesioning the arcuate nucleus (Martin et al., 1990) results in augmented fevers, thus supporting the necessity of α-MSH action in the brain to limit the magnitude and duration of fever.

The mechanism by which endogenously released α-MSH might act within the lateral septum to modulate fever is unknown, though it could involve an alteration of the thermoregulatory set point (Richards and Lipton, 1984) via neuroanatomical connections with thermoregulatory centres in the hypothalamus and preoptic area (Garris, 1979; Swanson and Cowan, 1979).

As mentioned earlier, α-MSH appears to be released into the septum during the rising (chill) phase of fever (Bell and Lipton, 1987). Maximal AVP release into the VSA, on the other hand, occurs during defervescence, the period following that

during which α-MSH is released. Hence, the timing of α-MSH and AVP release during fever appear to be complementary. Further, anatomical interconnections between the AVP and α-MSH endogenous antipyretic systems have been described (Mathieson et al., 1989b; Garris, 1979). Thus, based on this circumstantial evidence, it is possible that the two endogenous antipyretic systems may functionally interact with one anther. Future studies should therefore be directed at investigating this possibility.

E. VASOPRESSIN AS A CENTRAL NEUROTRANSMITTER

A requisite for identifying a role for AVP in endogenous antipyresis is that AVP fulfil the criteria of a neurotransmitter/neuromodulator, including the presence of AVP in the CNS, central synthesis of AVP, central release of AVP by neurons, central AVP receptors, central inactivation of AVP, and documentation of AVP actions on central neurons. Since the consideration of all neurotransmitter criteria is beyond the scope of this review, the following sections will examine central vasopressinergic pathways and receptor distribution only. Note, that as discussed elsewhere (Naylor, 1987), AVP meets most of the criteria required for identification of a neurotransmitter/neuromodulator within the VSA.

1) Central Vasopressinergic Pathways

a) Endocrine Vasopressinergic Pathways

Vasopressinergic soma of neurons serving endocrine functions have been identified in the paraventricular nucleus (PVN), the supraoptic nucleus and the hypothalamic accessory magnocellular groups. These neurons send projections to the

capillaries of the posterior pituitary, where AVP is released into the systemic circulation (Sofroniew, 1983a). In addition, PVN vasopressinergic projections to the portal capillaries of the median eminence have been documented (Sofroniew, 1983a).

Recently, vasopressinergic pathways which appear unrelated to endocrine functions have been documented. These pathways will be described in the following sections.

b) Nonendocrine Vasopressinergic Pathways

i) Cell Bodies

Immunohistochemical and lesion studies have identified the PVN, BST, and suprachiasmatic nucleus (SCN) as sites possessing high densities of vasopressinergic soma believed to be component parts of central nonendocrine pathways (Buijs et al., 1978; Buijs and Swaab, 1979; van Leeuwen and Caffé, 1983; De Vries and Buijs, 1983; Sofroniew and Weindl, 1978b). These studies also confirmed the less conspicuous presence of vasopressinergic soma in the medial amygdaloid nucleus (meA), supraoptic nucleus, locus coerulus, lateral septum, and dorsal medial hypothalamus (Buijs and Swaab, 1979; Sofroniew, 1983b; Caffé and van Leeuwen, 1983). All of the above regions stain for neurophysin II, hence indicating the synthesis of vasopressin in these perikarya.

In the next section, vasopressinergic pathways originating from the BST, PVN and SCN will be reviewed.

ii) Bed Nucleus of the Stria Terminalis

Current immunocytological evidence suggests that the primary target of

vasopressinergic neurons in the BST is the septal area where vasopressinergic nerve terminals have been identified (De Vries et al., 1985; van Leeuwen and Caffé, 1983). This is supported by lesion (De Vries and Buijs, 1983), electrophysiological (Disturnal et al., 1985) and retrograde transport studies (De Vries and Buijs, 1983) which also suggest that vasopressinergic fibres originating in the BST enter the septum through the diagonal band of Broca. Physiological evidence for this pathway also comes from the demonstration that electrical stimulation of the BST reduces fever via AVP release onto vasopressin receptors in the VSA (Naylor et al., 1988). Thus, the VSA receives its vasopressinergic input primarily from the BST.

The BST-VSA vasopressinergic projection appears to be under the influence of gonadal hormones since it was observed that following castration, both vasopressin-immunoreactive soma in the BST and vasopressin-immunoreactive fibres in the septal area were eliminated (De Vries et al., 1985). This suggestion has been confirmed by the localization of estrogen receptors on vasopressinergic soma of the BST using the double label method (Axelson and van Leeuwen, 1990). Related to this, Pittman et al. (1988) demonstrated that long-term castration of rats results in augmented fevers probably due to the elimination of AVP fibres within the VSA which originate from the BST.

Immunohistochemical and lesion studies have identified minor targets of BST vasopressinergic neurons, most notably the amygdala, the lateral habenula and the locus coerulus (van Leeuwen and Caffé, 1983; De Vries and Buijs, 1983).

iii) Paraventricular Nucleus

Immunocytochemical, retrograde transport, lesion, and electrophysiological

studies have described PVN vasopressinergic projections to a number of brainstem loci including the nucleus tractus solitarus, dorsal vagal nucleus, substantia nigra, mesencephalic grey, and raphe nuclei (De Vries and Buijs, 1983; van Leeuwen and Caffé, 1983; Sofroniew, 1980; Swanson, 1977; Buijs, 1978). Similar studies have identified additional PVN vasopressinergic projections to the diagonal band of Broca (Swanson, 1977), BST (De Vries and Buijs, 1983) and to the amygdala (Sofroniew, 1983b). Further, the PVN appears to be the primary source of vasopressin to the spinal cord (Pittman et al., 1981; Sofroniew, 1983b; Swanson, 1977; Buijs, 1978). Lastly, a PVN-septal vasopressinergic projection has been identified (Buijs, 1978), however, lesion and retrograde tracer studies do not confirm this finding (De Vries and Buijs, 1983).

In summary, PVN vasopressinergic neurons appear to project to diverse brain loci such as the amygdala, brainstem nuclei, BST, diagonal band of Broca, spinal cord, and possibly the septum.

iv) Suprachiasmatic Nucleus

Immunohistochemical and lesion studies provide evidence that the three principal targets of SCN vasopressinergic neurons are the organum vasculosum lamina terminalis (De Vries and Buijs, 1983; Buijs, 1978; Hoorneman and Buijs, 1982), the periventricular nucleus of the thalamus (Buijs, 1978; Hoorneman and Buijs, 1982) and the mediodorsal thalamus (Sofroniew and Weindl, 1978a; Hoorneman and Buijs, 1982). Other immunohistochemical studies have identified vasopressinergic projections to the medial amygdala originating from the SCN (Sofroniew, 1980; Sofroniew and Weindl, 1978a). The lateral septum has also been

identified as a target of SCN vasopressinergic neurons. However, lesion studies do not support this finding since septal AVP content remains unaltered following SCN lesioning (Hoorneman and Buijs, 1982; Buijs, 1978).

Immunocytochemical studies have identified minor targets of vasopressinergic neurons of the SCN. These include the diagonal band of Broca (Sofroniew and Weindl, 1978a; Sofroniew, 1980; Swanson, 1977), lateral habenula (Swanson, 1977; Buijs, 1978; Sofroniew and Weindl, 1978a), dorsal raphe nuclei (Swanson, 1977), mesencephalic grey (Swanson, 1977), and the hippocampus (Sofroniew, 1980; Swanson, 1977). The presence of these projections, however, has been called into question since lesion studies demonstrate that AVP levels in these sites do not change following SCN lesioning (Hoorneman and Buijs, 1982). However, since lesioning techniques are not entirely suitable for examining minor projections, these latter findings probably do not weigh heavily against the presence of the aforementioned minor SCN vasopressinergic projections.

In summary, SCN vasopressinergic neurons have several primary targets including the organum vasculosum lamina terminalis, periventricular nucleus of the thalamus, dorsal medial thalamus, and the medial amygdala.

2) Central Vasopressin Receptors

Vasopressin receptors have been well characterized in the periphery and 2 classes of receptors have been identified on the basis of ligand sensitivities and second messenger system activation (Mitchell et al., 1979; Brown et al., 1963; Kirk et al., 1979). Specifically, V_1 (vasopressor) receptors are characterized by low affinity vasopressin binding that is coupled to a Ca^{++}-dependant, cAMP-independent

mechanism. These receptors demonstrate the same ligand specificity as the receptors involved in vasopressor responses to vasopressin. On the other hand, V_2 (antidiuretic) receptors are characterized by high affinity AVP binding that is coupled to a cAMP-dependent, Ca^{++}-independent mechanism. These receptors demonstrate the same ligand specificity as receptors mediating antidiuretic responses to vasopressin.

Recently, Jard et al. (1986) identified a vasopressin receptor in the rat adenohypophysis that, like the V_1 receptor, is coupled to a Ca^{++}-dependant, cAMP-independent mechanism. However, this novel receptor possesses a ligand specificity that is neither vasopressor or antidiuretic in nature. Thus, this novel vasopressin receptor was classified as a V_{1b} receptor as opposed to the previously characterized vasopressor receptor which is now known as a V_{1a} receptor.

a) Distribution

Autoradiographic and radioligand binding studies provide evidence for the presence of vasopressin binding sites in the PVN and supraoptic nucleus as well as in extrahypothalamic sites such as the amygdala, septum, nucleus of the tractus solitarus, hippocampus, and the olfactory nucleus (Yamamura et al., 1983; Dorsa et al., 1983; Pearlmutter et al., 1983; Biegon et al., 1984; van Leeuwen and Wolters, 1983; Baskin et al., 1983; Dorsa et al., 1984). In general, this labelling corresponds well with the central distribution of vasopressinergic terminals and with known sites of AVP action.

b) Types

Pharmacological studies, using peripherally active agonists and antagonists, have revealed that central vasopressin receptors in the ventral septum, amygdala, hippocampus and lateral septum resemble the vasopressor (V_{1a}) subtype (Jard et al., 1986; Poulin et al., 1988). Support for this also comes from physiological studies using similar pharmacological tools. It appears that most central vasopressin receptors are not similar to antidiuretic (V_2) receptors since central injections of V_2 antagonists do not block AVP effects on convulsive behaviour (Naylor et al., 1985; Burnard et al., 1986) or septal antipyresis (Cooper et al., 1987; Naylor et al., 1988). However, one study has provided evidence suggesting that V_2 receptors may mediate AVP effects on neuronal activity in the supraoptic nucleus (Abe et al., 1983).

Vasopressor antagonists, on the other hand, have been shown to block numerous central AVP actions. For example, the V_{1a} antagonist, $d(CH_2)_5$Tyr(Me)AVP (Kruszynski et al., 1980), has been shown to block AVP effects on hippocampal neuronal firing (Tiberiis et al., 1983), behavioural convulsive activity (Naylor et al., 1985; Burnard et al., 1986), cardiovascular regulation (Matsuguchi et al., 1982) and septal antipyresis (Naylor et al., 1988). These results have been taken to support the V_{1a} nature of central vasopressin receptors at these sites. Such an interpretation must be met with caution, however, since AVP could be cleaved into fragments that interact with a novel receptor that binds to a particular AVP fragment as well as $d(CH_2)_5$Tyr(Me)AVP. Thus, blocking an effect with vasopressor antagonists can only implicate a V_{1a}-like receptor mechanism since a novel receptor with the aforementioned characteristics could be involved. This caution is warranted since an AVP derivative ($[pGlu_4,Cyt_6]AVP_{4-8}$) has central behavioural effects, no

peripheral effects but can be blocked by a vasopressor antagonist (De Weid et al., 1984). Further, Kovacs and De Weid (1983) have shown that a particular fragment of AVP is a potent activator of a behavioural response while it is ineffective as an antipyretic.

In summary, there exists evidence demonstrating the central presence of V_{1a}-like receptors and to a lesser extent, V_2-like receptors. V_{1a}-like receptors appear to be located in the septum, amygdala, nucleus of the tractus solitarus, hippocampus, and the olfactory nucleus.

F. THE AMYGDALA

The amygdala is a multinuclear limbic structure consisting of more the 16 nuclei that can be organized into 4 groups on the basis of histochemistry and connectivity, namely, the olfactory amygdala, the central amygdala, the basolateral amygdala, and the medial amygdala (de Olmos et al., 1985). There exists evidence implicating the amygdala in general thermoregulatory processes as well as endogenous antipyresis. This evidence will be reviewed in the following sections.

1) The Amygdala and Thermoregulation

Evidence has been adduced implicating a role for the amygdala in thermoregulation as a centre for the processing and relaying of thermal information. For example, it was shown that electrical stimulation of the medial or lateral amygdala causes a rise in body temperature (Koigegami et al., 1952; Kawakami et al., 1972), whereas lesioning the amygdala and its neighbouring area, results in a fall in body temperature (Anand and Brobeck, 1952). In addition, lesioning the

amygdala significantly alters the relationship between rectal temperature and respiratory rate (Ogata and Murakami, 1972). Further, Kawakami et al. (1972) demonstrated that changes in EEG recordings from the amygdala occur during heat and cold exposure and that these changes decrease upon acclimation to the thermal challenge. Thus, from these findings it appears that thermal input from the skin might pass through the amygdala in its processing and that stimulating or lesioning the amygdala alters this.

More recent electrophysiological studies identified thermoresponsive neurons in the VSA which are excited by scrotal heating and are inhibited by electrical stimulation of the medial amygdaloid nucleus (Disturnal et al., 1986). Thus, the hypothesis that the amygdala plays a role in temperature regulation appears to be supported by this finding, however, it is possible that the testicular afferents passing through the amygdala subserve a reproductive rather than a thermoregulatory function.

2) The Amygdala and Endogenous Antipyresis

a) Vasopressin Release

It has recently been suggested that vasopressin might act within the amygdala as part of an endogenous antipyretic system. For example, Kasting and Martin (1983) have shown by radioimmunoassay that AVP levels in the amygdala decrease during endotoxin fever in rats and these changes were suggested to indicate increased release of AVP into this site. Other studies have shown increased immunoreactivity for AVP in amygdalar nerve terminals and paraventricular soma during fever in the nonpregnant guinea-pig (Zeisberger et al., 1983; Zeisberger et

al., 1986) as well as in the pregnant guinea-pig at term, a time when the animal is refractory to endotoxin fever (Merker et al., 1980; Zeisberger et al., 1981). There is also immunocytochemical evidence for an increase in AVP content in amygdalar nerve terminals and paraventricular soma during the development of tolerance to the pyrogen poly I:poly C (Cooper et al., 1988). Additionally, cold-adaptation has been found to be associated with a reduced febrile response to bacterial pyrogen along with increased immunoreactive AVP levels in supraoptic and paraventricular soma as well as septal and amygdalar nerve terminals (Merker et al., 1989). An increase in immunoreactive AVP levels in nerve terminals or soma, however, may not necessarily indicate increased synthesis or release of the peptide. In fact these findings could indicate a reduced release of AVP in the face of steady synthesis or combinations of both altered synthesis or release. Nonetheless, a change in immunoreactivity for AVP in amygdalar nerve terminals suggests some as yet unknown function of this region during fever.

b) Vasopressingeric Input

Anatomical studies have demonstrated the presence of vasopressinergic nerve terminals throughout the entire amygdaloid complex with the medial amygdaloid nucleus (meA) being the most densely innervated (De Vries et al., 1985; Buijs and Swaab, 1979; Sofroniew and Weindl, 1978b; Sofroniew, 1983a; Zimmerman et al., 1984; Sofroniew, 1983b). To date, four putative sources of AVP to the amygdaloid complex have been identified, namely, the PVN, BST, SCN and meA. The PVN appears to send vasopressinergic projections to the entire amygdaloid complex (Buijs, 1978; Sofroniew, 1980), whereas SCN vasopressinergic neurons project to the medial

amygdala only (Sofroniew, 1980; Sofroniew and Weindl, 1978a). The SCN projection, however, has been called into question since lesioning the SCN leaves vasopressinergic fibres in the medial amygdala unaltered (Hoorneman and Buijs, 1982).

Recently, it has been demonstrated that following castration, vasopressin levels (determined immunocytochemically) decrease in soma in the BST and meA as well as in nerve terminals in the amygdaloid complex and VSA (De Vries et al., 1985; see earlier). From this observation, two suggestions were made. The first is that vasopressinergic soma in the BST and/or meA may project to the entire amygdaloid complex. Second, this vasopressinergic projection might be under the influence of gonadal hormones. Supporting this is the demonstration of the presence of estrogen receptors on vasopressinergic soma in the BST and meA using the double labelling method (Axelson and van Leeuwen, 1990). As discussed previously, Pittman et al. (1988) demonstrated that the long-term castration of rats results in enhanced fevers, presumably by decreasing AVP levels in septal nerve terminals. Since castration also eliminates virtually all the AVP content of amygdalar nerve terminals, it is possible that AVP elimination in this site may have contributed to the observed enhancement of febrile responses following castration. This possibility should be investigated further.

c) Vasopressin Receptors

The presence of vasopressin binding sites within the amygdala has been widely documented. Several studies report the presence of strong vasopressin binding in the central amygdaloid nucleus and surrounding areas (Lawrence et al.,

1988; Biegon et al., 1984; Tribollet et al., 1988; Dorsa et al., 1984; Freund-Mercier et al., 1988a; Freund-Mercier et al., 1988b) along with moderate vasopressin binding in the medial and basolateral amygdaloid nuclei (Dorsa et al., 1984; Freund-Mercier et al., 1988a; Freund-Mercier et al., 1988b). These vasopressin binding sites appear to resemble the vasopressor (V_{1a}) receptor based on binding kinetics and ligand sensitivity (Dorsa et al., 1984; Tribollet et al., 1988; Lawrence et al., 1988).

Based on findings reviewed in this and the previous section, it appears that amygdalar vasopressin binding sites do not correlate well with the distribution of vasopressinergic nerve terminals. Specifically, while the entire amygdaloid complex appears to receive vasopressinergic input, vasopressin binding sites have not been observed in all amygdalar regions. Furthermore, while the vasopressinergic input to the meA is strong compared to the remainder of the amygdala, vasopressin binding at this site is moderate and has not been consistently observed. The cause of these discrepancies is unknown although it is possible that novel vasopressin receptors might exist within the amygdala that cannot be consistently identified.

d) Anatomical Connections

In the next sections, anatomical connections of the four amygdalar groups will be reviewed focusing on connections with thermoregulatory centres. For clarity, only the principal connections of each amygdalar group will be examined.

i) Olfactory Amygdala, Central Amygdala and Basolateral Amygdala

The olfactory amygdala, as the name implies, plays an important role in olfaction (de Olmos et al., 1985). The olfactory amygdala is characterized by

extensive reciprocal connections with sites receiving olfactory input such as the olfactory bulb (Haberly and Price, 1978; Luskin and Price, 1983) as well as with the hippocampal formation (Luskin and Price, 1983; Wyss, 1981). This connectivity implicates the olfactory amygdala in olfaction as well as learning of some kind. It appears, however, that the olfactory amygdala is not extensively interconnected with thermoregulatory centres, though a few scattered connections have been documented (de Olmos et al., 1985).

The basolateral and central amygdala, like the olfactory amygdala, have only minor connections with thermoregulatory centres (de Olmos et al., 1985). The basolateral amygdala is characterized by extensive afferents to the striatum (Kelly et al., 1982; Krettek and Price, 1978) in addition to major reciprocal connections with cortical structures such as the puriform cortex, agranular insular cortex and prelimibic cortex (Beckstead, 1979; Krettek and Price, 1977; Divac, 1979; Ottersen, 1982). The central amygdala, on the otherhand, is extensively interconnected with brain stem nuclei involved in cardiovascular regulation and other autonomic processes (Ottersen, 1981; Schwaber et al., 1982; Takayama et al., 1990).

ii) Medial Amygdala/Medial Amygdaloid Nucleus

Of the four amygdalar groups, the medial amygdala possesses the most extensive connections with key thermoregulatory centres. The medial amygdala consists of the meA, the posteromedial portion of the substantia innominata, as well as the medial and lateral divisions of the BST (de Olmos et al., 1985). Interestingly, the meA occupies a central position in the medial amygdala since it serves as a gateway for thalamic afferents to all other medial amygdalar nuclei as well as a

gateway for brainstem efferents (Ottersen and Ben-Ari, 1979; Veening, 1978). In addition, the meA possesses extensive reciprocal connections with sites involved in fever, antipyresis, and general thermoregulatory processes (de Olmos et al., 1985).

The meA is reciprocally connected with the BST (Caffé et al., 1987; Swanson and Cowan, 1979; Weller and Smith, 1982; Ottersen, 1980) and PVN (Sawchenko and Swanson, 1983; Tribollet and Dreifuss, 1981; Caffé et al., 1987; Pittman et al., 1981), both of which are putative sources of vasopressin to the VSA and the amygdaloid complex. Further, reciprocal connections with the arcuate nucleus (the sole source of extrahypothalamic α-MSH) have been identified by immunohistochemical and retrograde tracer studies (de Olmos et al., 1985; Mathieson et al., 1989a; Ottersen, 1980; Krieger et al., 1979). Thus, the meA is interconnected with several sources of putative endogenous antipyretics, therefore implicating a role for this site in endogenous antipyresis.

The meA is also closely interconnected with other sites important in fever, antipyresis, and general thermoregulatory processes. These include the ventromedial (Saper et al., 1976; Krettek and Price, 1978; Luiten et al., 1983), lateral (Veening, 1978; Ottersen, 1980; Ottersen and Ben-Ari, 1979) and dorsomedial (de Olmos and Carrer, 1978) hypothalamus, as well as the VSA (Caffé et al., 1987; Disturnal et al., 1985; Mathieson et al., 1989a), and POAH (Swanson, 1976; Conrad and Pfaff, 1976a; Ottersen, 1980; Caffé et al., 1987). In summary, although the amygdala is a multinuclear structure, anatomical evidence suggests that the meA may be selectively involved in fever, antipyresis and general thermoregulatory processes.

G. RATIONALE FOR RESEARCH

There seems to be enough evidence to implicate the amygdala (specifically the meA) in fever and AVP-induced antipyresis, but that role remains to be clarified. Experiments were therefore designed to achieve the following objectives:

1) To determine if AVP is antipyretic when exogenously administered within the meA.
2) To characterize the central receptor mediating AVP-induced antipyresis in the meA.
3) To determine if the action of endogenously released AVP is necessary in the meA to modulate body temperature during fever.

CHAPTER 2

ANTIPYRETIC ACTION OF VASOPRESSIN IN THE MEDIAL AMYGDALOID NUCLEUS OF THE CONSCIOUS RAT

A. INTRODUCTION

There exists considerable evidence suggesting that vasopressin may act within the VSA as an endogenous antipyretic in the rat (Cooper, 1987). Recently, however, it has been suggested that AVP may also act within the amygdala as an endogenous antipyretic. Specifically, AVP release into the amygdala has been demonstrated to occur during fever (Kasting and Martin, 1983; Zeisberger et al., 1983; Zeisberger et al., 1986) and during the development of pyrogen tolerance in animals at term (Merker et al., 1980; Zeisberger et al., 1981) or animals subjected to repeated administration of pyrogen (Cooper et al., 1988). Additionally, increased AVP levels in the amygdala have been observed in cold-adapted animals that are refractory to endotoxin.

Although the amygdala is a multinuclear limbic structure, evidence suggests that the medial amygdaloid nucleus (meA) could be selectively involved in AVP-induced antipyresis. First, vasopressinergic nerve terminals have been identified within the meA (Buijs and Swaab, 1979; De Vries et al., 1985). Second, AVP binding sites have been identified in the meA and surrounding areas that appear to resemble the V_{1a} subtype (Freund-Mercier et al., 1988b; Dorsa et al., 1984; Lawrence et al., 1988). Third, anatomical and electrophysiological studies have shown that meA possesses afferent and efferent connections with sites important in fever and antipyresis including the POAH (Swanson, 1976; Conrad and Pfaff, 1976a; Ottersen, 1980; Sperk et al., 1983; Caffé et al., 1987), VSA (Caffé et al., 1987; Disturnal et al.,

1985; Mathieson et al., 1989a), arcuate nucleus (de Olmos et al., 1985; Mathieson et al., 1989a; Ottersen, 1980; Krieger et al., 1979), PVN (Sawchenko and Swanson, 1983; Tribollet and Dreifuss, 1981; Caffé et al., 1987; Pittman et al., 1981; Sperk et al., 1983), and BST (Caffé et al., 1987; Swanson and Cowan, 1979; Weller and Smith, 1982; Ottersen, 1980).

Taken together, these findings imply that AVP might act within the meA to modulate body temperature during fever. The present experiments were therefore carried out to determine if the exogenous administration of AVP into the meA could attenuate PGE and IL-1 fevers in the conscious rat.

B. METHODS

Sixty one male Sprague-Dawley rats initially weighing 250-280 g were used. The animals were housed in a colony room maintained at 22 ± 1°C on a 12 h light-dark cycle with continuous access to food and water.

Under sodium pentobarbitol anaesthesia (Somnotol; 65 mg/kg i.p.), 23-gauge (thin-wall) stainless steel guide cannulae were bilaterally implanted into each rat using stereotaxic procedures (Paxinos and Watson, 1982) so that the tips remained 5 mm above the intended site of injection into the meA. In addition, 20-gauge stainless steel guide cannulae were similarly positioned over the lateral cerebral ventricles so that the tips rested on dura mater. Three stainless steel anchor screws were inserted into the calvarium and the cannulae were secured in place with cranioplast cement. Stylets were inserted to occlude the cannulae when they were not in use. Paraffin-coated temperature-sensitive radiotransmitters (Mini Mitter Inc.) were intraperitoneally implanted into the animals for the future remote monitoring

of core temperature. Following surgery, the animals were allowed a 10 day recovery period.

All animals used in this study were subjected to 2 experimental trials, the order of which was counterbalanced and separated by at least 5 days. All experimental trials were carried out between 0800 - 1600 h, the time when body temperature in the rat is relatively stable (Malkinson et al., 1990). On each experimental test day, rats were placed individually into plastic cages without restraint and allowed a 1 hr settling period. Following this settling period, baseline recordings of core temperature were made for 1 h prior to the i.c.v. injection of IL-1, PGE_1, or aCSF (see later). Core temperature was monitored by antennae designed to receive the transmission of the intraperitoneally implanted temperature-sensitive radiotransmitters. Core temperature was recorded on-line (Dataquest III, Data Sciences Inc.) at 5 min intervals using a Heath AT computer system.

To microinject IL-1 (50 U, R & D Systems Inc.), a sterile 25-gauge stainless steel injector needle was lowered into the left lateral cerebral ventricle. The needle was connected by a length of PE-20 tubing filled with IL-1 (10 U/μl) and 5 μl of this solution was injected i.c.v. by gravitational flow over 45 seconds. Stock IL-1 solution (200 U/ml in PBS containing 0.1% BSA) was kept at 4°C and final dilution was made immediately prior to experimentation using pyrogen-free artificial cerebrospinal fluid (aCSF: composition in mM: 26.0 $NaHCO_3$; 2.0 $MgSO_4$; 1.5 $CaCl_2$; 124.0 NaCl; 1.8 KCl; 1.3 KH_2PO_4; 1.0 glucose). Following the completion of injection, the injector needle was left in place for one additional minute to prevent any solution from being drawn up along the needle tract when it was removed. In the event that gravitational flow into the left lateral cerebral ventricle

could not be obtained, the injection was made into the right lateral cerebral ventricle.

PGE_1 (50 ng, Bachem) was injected i.c.v. in the same manner as IL-1. Stock PGE_1 solution (2.0 mg/ml in absolute EtOH) was kept at -20°C in the dark and final dilution was made immediately prior to experimentation using pyrogen-free aCSF.

The experimental animals also received injections of AVP (40 pmol in 1μl, Bachem) or vehicle (aCSF) bilaterally directed toward the meA over a 1 min period. To achieve this, sterile 27-gauge injector cannulae were lowered through the guide cannulae to the level of the meA. The injector cannulae were connected by a length of PE-20 tubing to gas tight 10 μl glass syringes mounted on a Harvard infusion pump that had been previously calibrated to deliver 1 μl/min. Following the completion of injection, the injector cannulae were left in place for one additional minute to prevent any solution from being drawn up along the injector cannula tracts when they were removed. AVP solution was prepared immediately prior to each experiment from a stock solution (10^{-3} M in 0.025 M acetic acid) that was stored at 4°C.

Series I and II

In first series of experiments, 13 animals were subjected to two experimental trials each. In one experimental trial, each rat received an i.c.v. injection of IL-1 (50 U/5μl) followed 150 min later by a bilateral injection of AVP (40 pmol/1μl per side) that was directed toward the meA. In the other experiment trial, each rat received an i.c.v. injection of IL-1 (50 U/5μl) followed 150 min later by a bilateral injection

of vehicle (aCSF: 1μl per side) into the meA region.

In the second series of experiments, 12 animals were used. In one trial, each rat received an i.c.v. injection of aCSF (5 μl) followed 150 min later by a bilateral injection of AVP (40 pmol/1μl per side) that was directed toward the meA. In the other experiment trial, each rat received an i.c.v. injection of aCSF (5μl) followed 150 min later by a bilateral injection of aCSF (1μl per side) into the meA region.

Series III and IV

In the third series of experiments, 18 animals were subjected to two experimental trials each. In one trial, each rat received an i.c.v. injection of PGE_1 (50 ng/5μl) that was preceded by 5 min by a bilateral injection of AVP (40 pmol/1μl per side) that was directed toward the meA. In the other trial, each rat received an i.c.v. injection of PGE_1 (50 ng/5μl) that was preceded by 5 min by a bilateral injection of aCSF (1μl per side) into the meA region.

In the fourth series of experiments, 18 animals were used. However, in one animal, the headplate (the array of guide cannulae bonded by cranioplast cement) became dislodged, thus only 17 animals were subjected to two experimental trials each. In one trial, each rat received an i.c.v. injection of aCSF (5μl) that was preceded by 5 min by a bilateral injection of AVP (40 pmol/1μl per side) that was directed toward the meA. In the other trial, each rat received an i.c.v. injection of aCSF (5μl) that was preceded by 5 min by a bilateral injection of aCSF (1μl per side) into the meA area.

Upon completion of all experiments, each rat was anaesthetized deeply with sodium pentobarbitol. Tissue injection sites were marked by 1 μl injections of

Pelican Special black ink. The brain was perfused with saline followed by neutral formalin by retrograde injection into the aorta. The brain was then removed and blocked in the coronal plane and sectioned at 60 μm on a sledge microtome. Each section was stained with neutral red and tissue injection sites were localized by light microscopy.

All animals used in this study were separated into two groups for data analysis, namely, those receiving tissue injections into the meA on at least one side of the brain, and those receiving tissue injections into sites surrounding, but not impinging upon the meA on both sides of the brain. Temperature data were presented as deviations from the mean baseline core temperature recorded for 1 hr prior to the i.c.v. injection of IL-1, PGE_1 or aCSF. In this study, this time was taken to represent time 0. For animals in the first two series of experiments, the maximal drop in core temperature was determined for the period immediately following the tissue injection of AVP or aCSF. This value was calculated by subtracting the lowest core temperature recorded following tissue injection from the average core temperature recorded for 15 minutes prior to this injection. All data were subjected to a one way Analysis of Variance followed by Newman Keuls post-hoc comparisons. For all tests, statistical significance was set at $P < 0.05$.

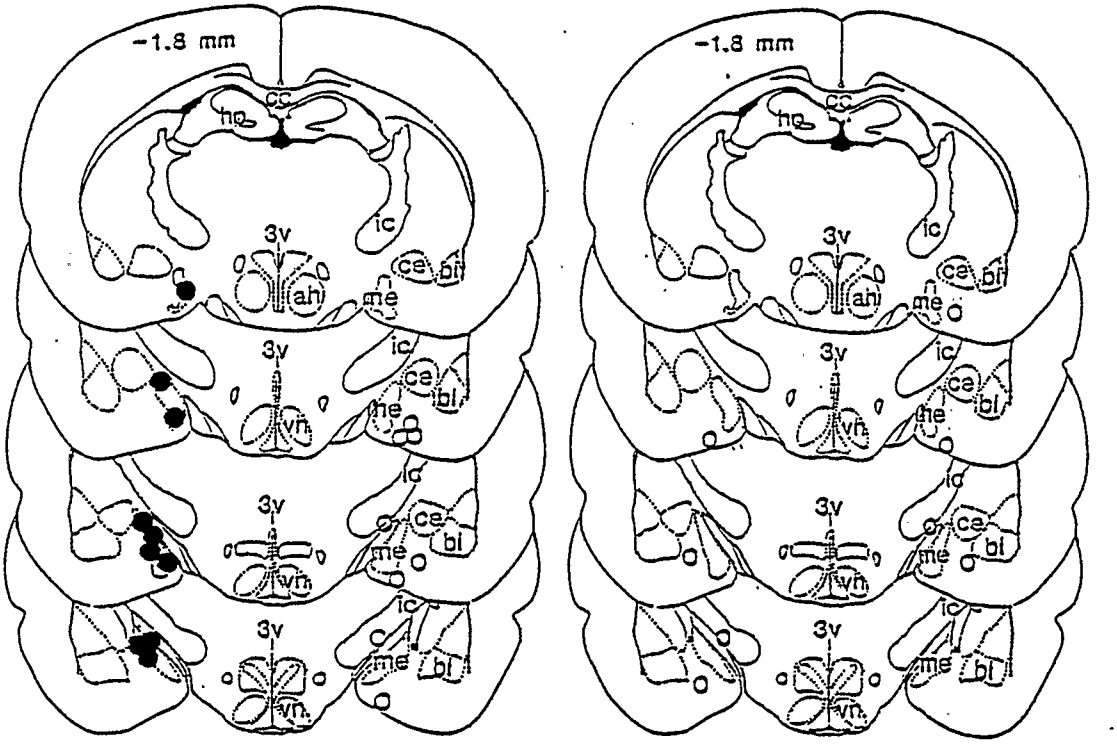
C. RESULTS

Series I and II

Histological examination of tissue injection sites of the 25 animals used in the first two series of experiments showed that tissue injections were localized to the meA and adjacent areas (Fig 2). As previously mentioned, animals were separated

Fig. 2. Schematic histological sections of the rat forebrain showing injection sites that were localized to the meA (filled circles) or to sites surrounding but not impinging upon the meA (open circles) for animals in the first (upper panel) or second (lower panel) series of experiments. Note that histological data obtained from animals in which tissue injection was localized to the meA on at least one side of the brain are summarized in the left side of both panels. Histological data from animals receiving injections outside the meA on both sides of the brain are summarized in the right side of both panels. For clarity, injection sites localized to the meA are placed on one side of the appropriate histological sections. Abbreviations: 3v, third ventricle; ah, anterior hypothalamus; bl, basolateral amygdaloid nucleus; cc, corpus callosum; ce, central amygdaloid nucleus; hp, hippocampal area; ic, internal capsule; me, medial amygdaloid nucleus; vh, ventromedial hypothalamus.

Series I



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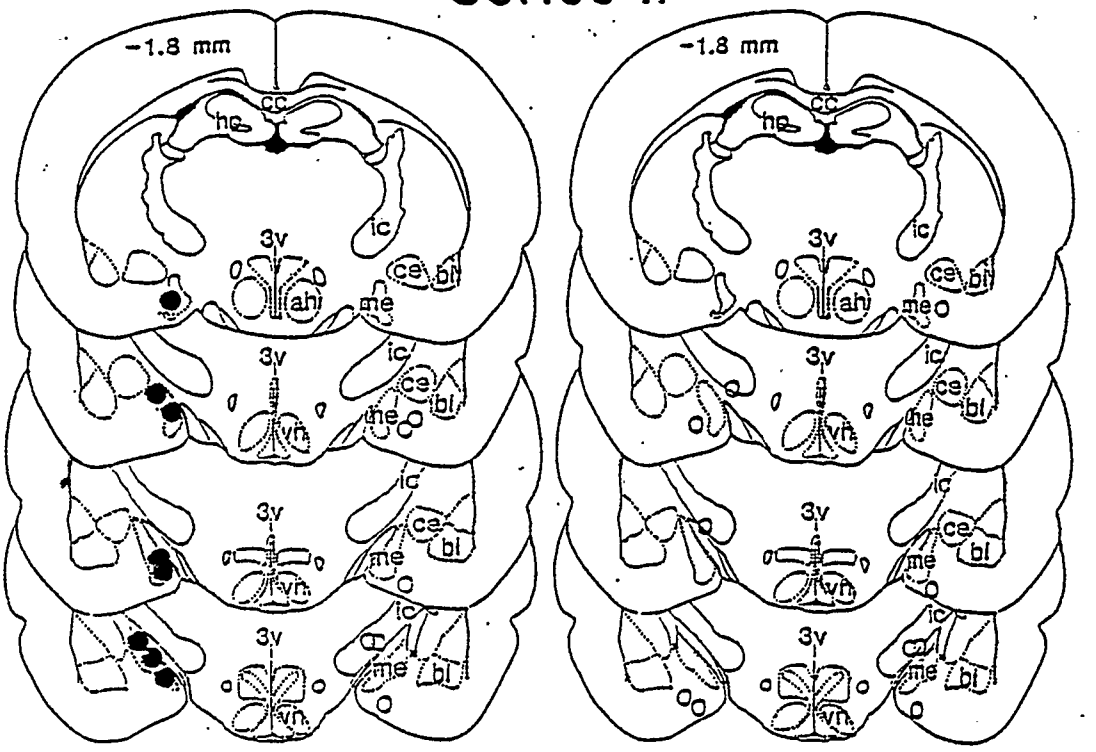


on the basis of whether or not tissue injection was localized to the meA on at least one side of the brain.

The results from experimental trials carried out on a single animal in the first experimental series are presented in Fig. 3. Intracerebroventricular injection of IL-1 (50 U) evoked an immediate rise in core temperature from baseline values of 37.3 ± 0.0°C (Fig. 3, top panel) and 37.0 ± 0.0°C (Fig. 3, bottom panel) to a plateau of 1.6 - 1.9°C above baseline for this animal. Further, injection of AVP (40 pmol) into the meA of this febrile animal caused an immediate, but transient 0.8°C drop in core temperature lasting approximately 20 minutes (Fig. 3, top panel). Similar injection of vehicle (aCSF; 1μl) into the meA, on the other hand, elicited a comparatively small 0.2°C drop in core temperature (Fig. 3, bottom panel).

The results from experimental trials carried out on a single animal in the second series of experiments are presented in Fig. 4. Following i.c.v. injection of aCSF (5μl) there were modest changes in core temperature from baseline values of 37.0 ± 0.1°C (Fig. 4, top panel) and 36.9 ± 0.0°C (Fig. 4, bottom panel). Further, injection of AVP into the meA of this afebrile animal was followed by a 0.3°C drop in core temperature (Fig. 4, top panel). Following similar injection of aCSF into the meA of the same animal was a 0.1°C drop in core temperature (Fig. 4, bottom panel).

Fig. 5 summarizes the results of the first and second experimental series from animals in which tissue injections were localized to the meA. As shown in Fig. 5, aCSF injection into the meA of animals made febrile by IL-1 was followed by a 0.3 ± 0.0°C drop in core temperature (core temperature at time of aCSF injection = 1.8 ± 0.1°C above baseline; n = 9). Similar injection of AVP into the meA,

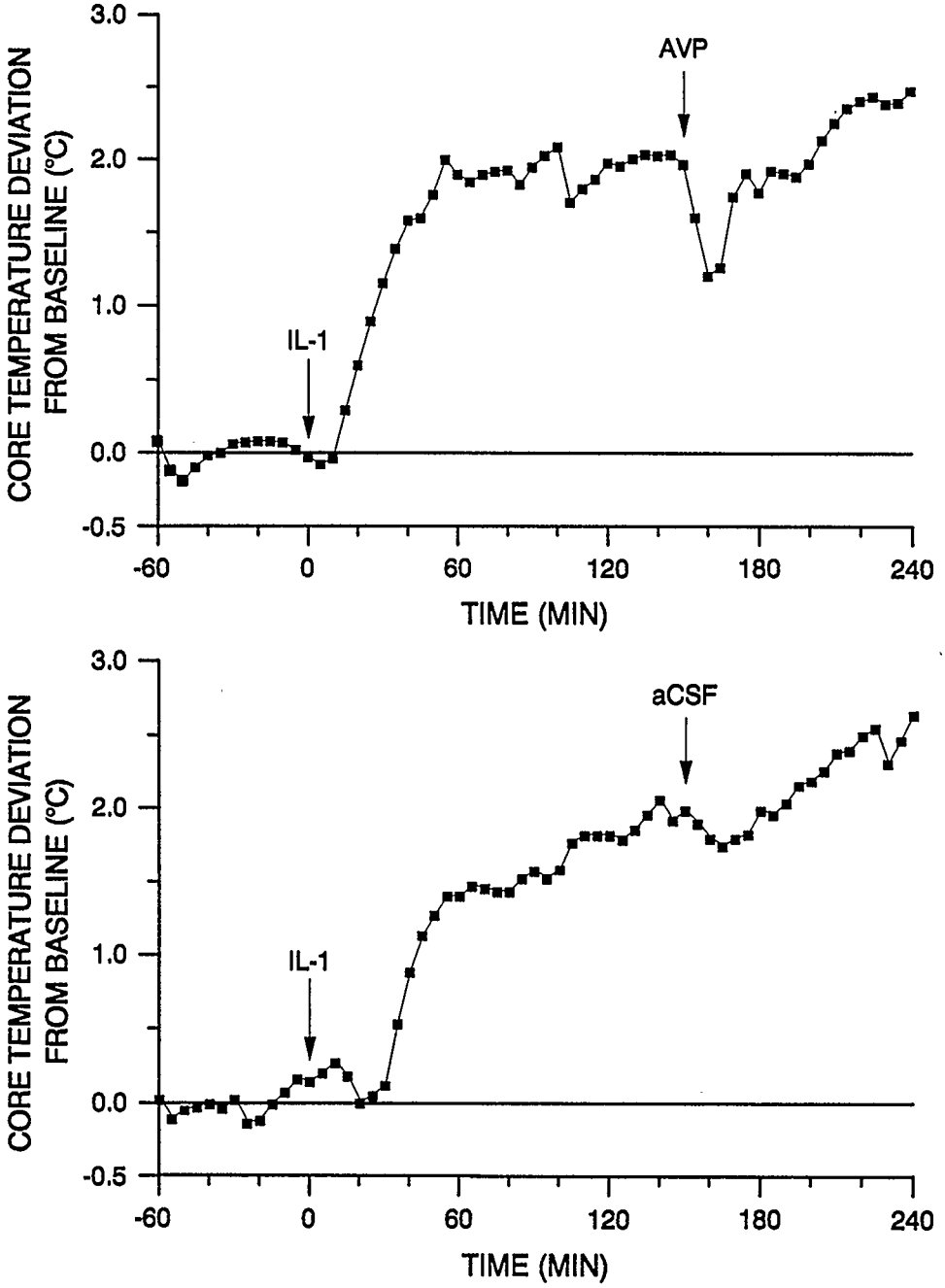


Fig. 3. Core temperature response to the i.c.v. injection of IL-1 in a single animal. One hundred and fifty minutes following this, either AVP (top panel) or aCSF (bottom panel) were bilaterally injected into the meA.

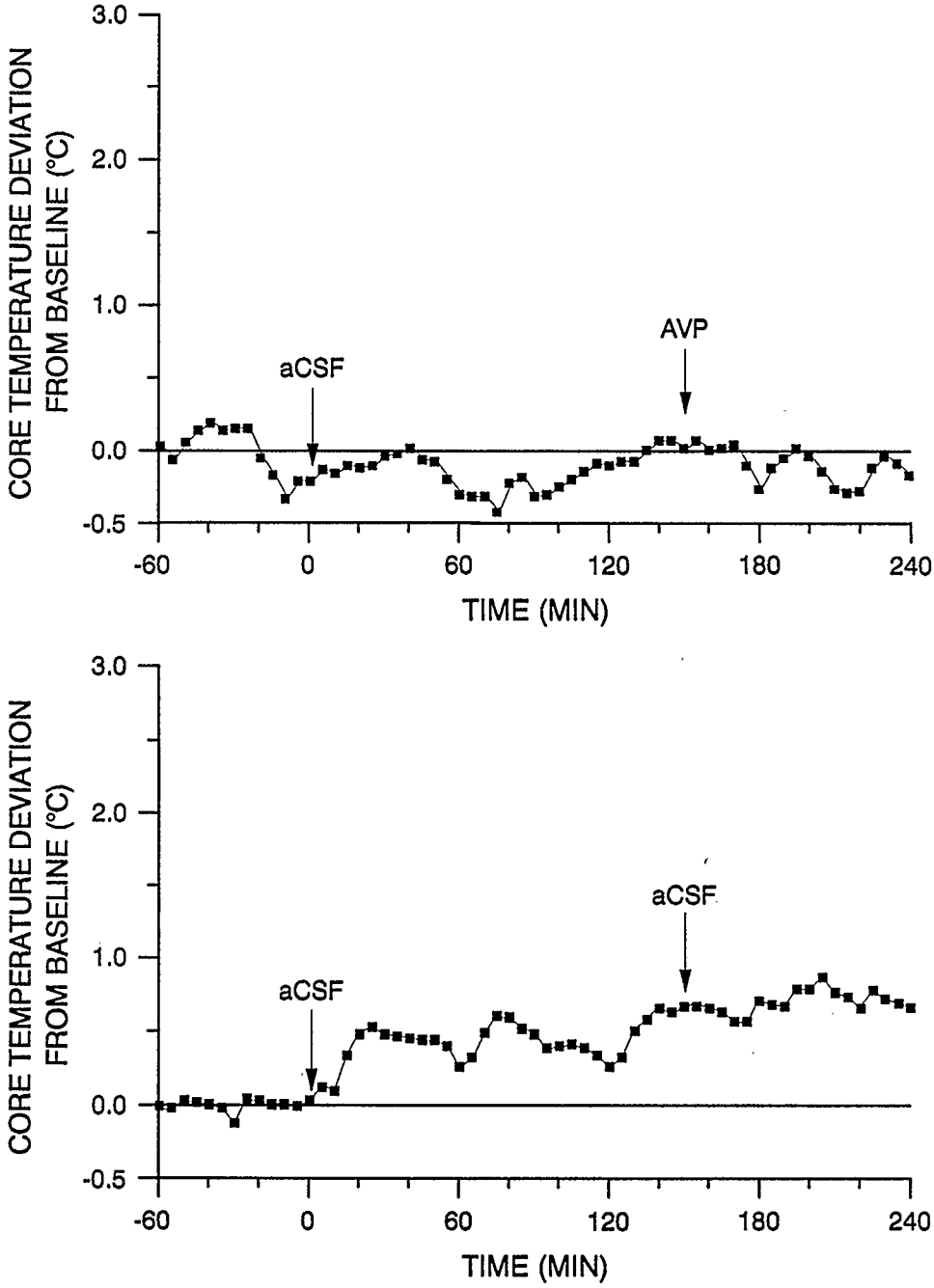


Fig. 4. Core temperature response to the i.c.v. injection of aCSF in a single animal. One hundred and fifty minutes following this, either AVP (top panel) or aCSF (bottom panel) were bilaterally injected into the meA.

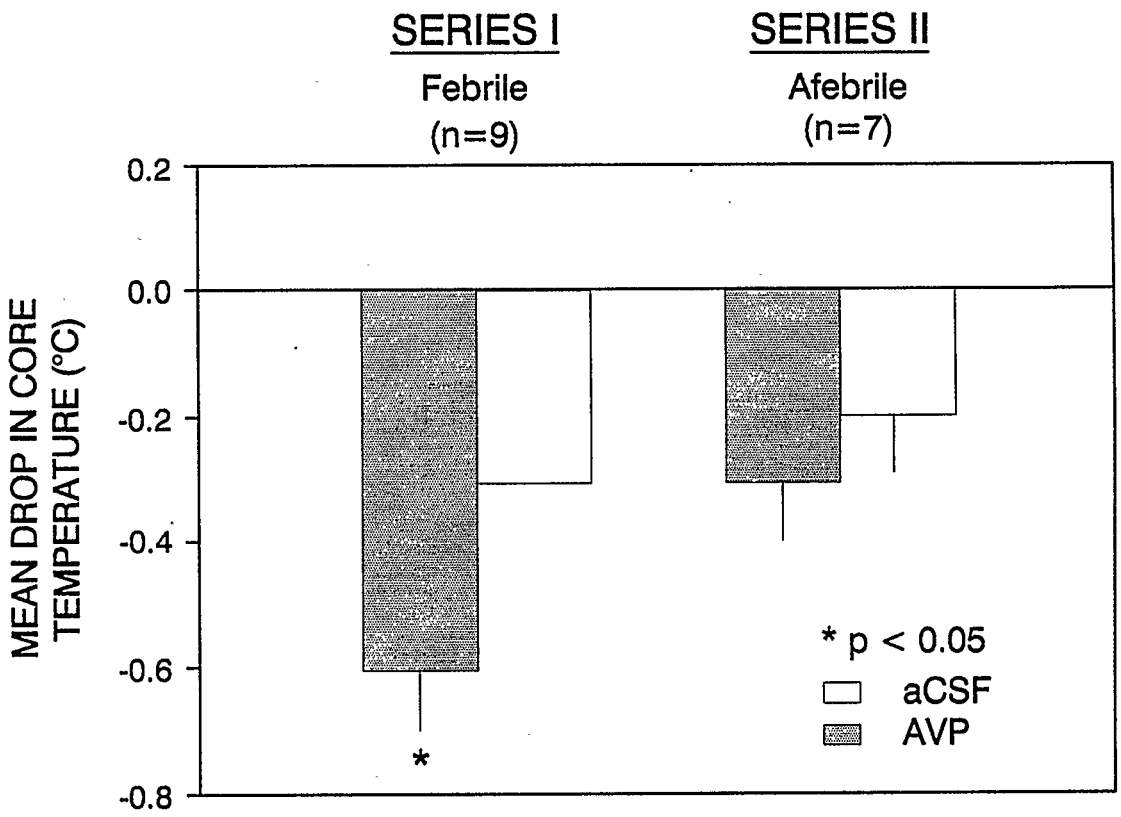


Fig. 5. Summary of mean (± S.E.M) maximal core temperature drops following the bilateral injection of AVP (filled bars) or aCSF (open bars) into the meA of febrile or afebrile animals (*P < 0.05).

however, evoked a significantly larger drop in core temperature of 0.6 ± 0.1°C (core temperature at time of AVP injection = 1.9 ± 0.1°C above baseline; n = 9; P < 0.05; Fig. 5). The injection of aCSF into the meA of afebrile animals was followed by a 0.2 ± 0.1°C drop in core temperature (core temperature at time of aCSF injection = 0.6 ± 0.1°C above baseline; n = 7; Fig. 5). Following similar injection of AVP into the meA was a 0.3 ± 0.1°C drop in core temperature that was not significantly different compared to control injection of aCSF (core temperature at time of AVP injection = 0.5 ± 0.1°C above baseline; n = 7; P > 0.05; Fig. 5).

Fig. 6 summarizes the results obtained from animals in the first two experimental series in which tissue injections were localized to sites surrounding, but not impinging upon the meA. Vasopressin injections into sites surrounding the meA of febrile or afebrile animals did not evoke significant drops in core temperature compared to their aCSF controls (Febrile, maximal temperature drop; AVP = 0.3 ± 0.1°C; aCSF = 0.2 ± 0.1°C; n = 4; P > 0.05. Afebrile, maximal temperature drop; AVP = 0.3 ± 0.1°C; aCSF = 0.2 ± 0.1°C; n = 5; P > 0.05; Fig. 6).

Series III and IV

Histological examination of tissue injection sites of the 35 animals used in the third and fourth series of experiments showed that tissue injections were localized to the meA and to adjacent areas (Fig 7). As mentioned previously, animals were separated on the basis of whether or not tissue injections were localized to the meA on at least one side of the brain.

Fig. 8 summarizes the results obtained from animals in the third and fourth series of experiments in which tissue injections were localized to the meA. From an

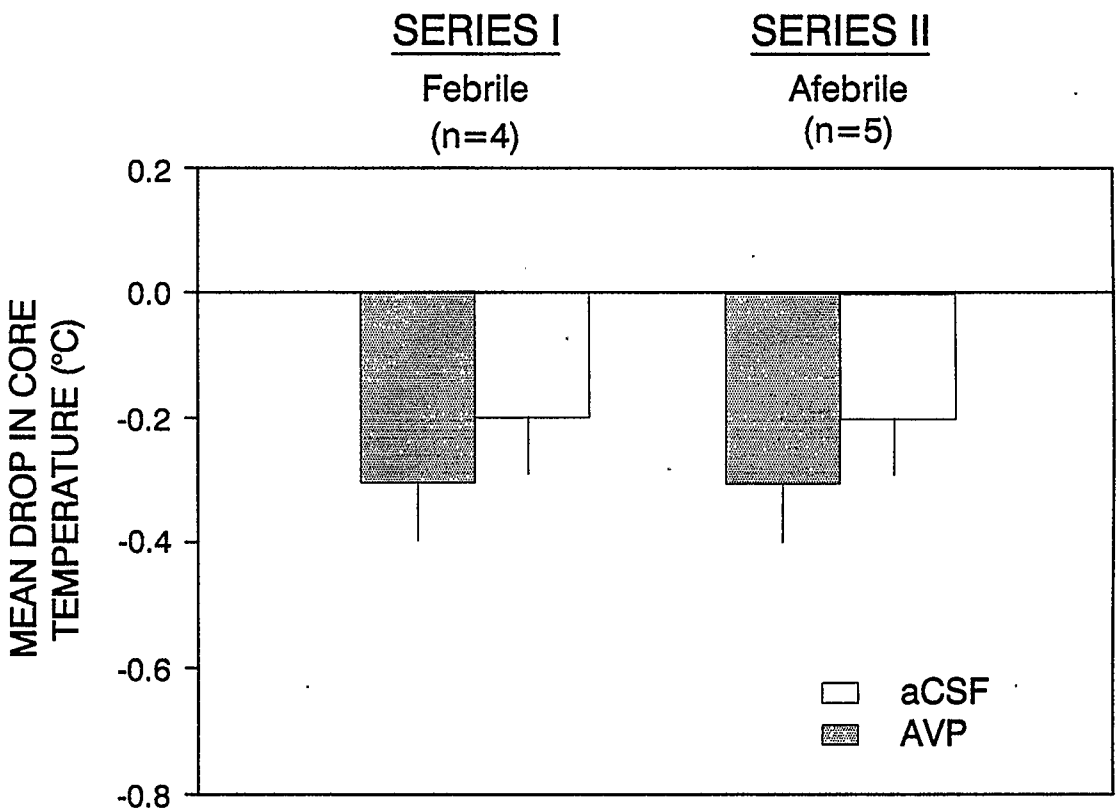
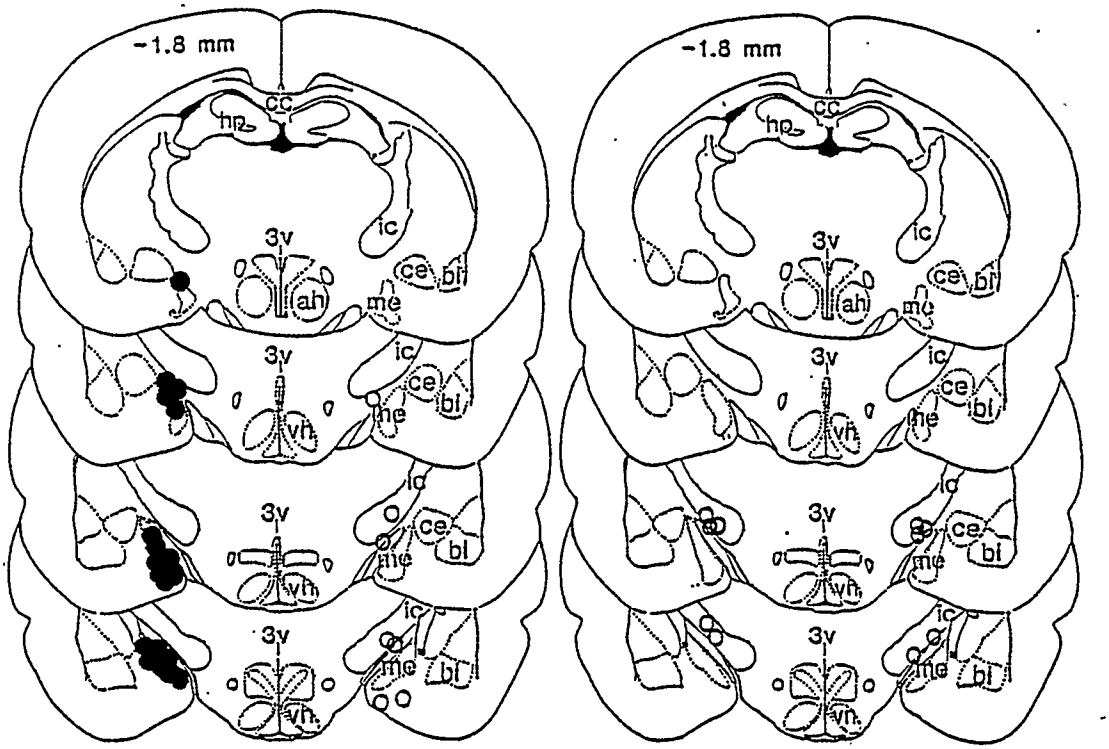


Fig. 6. Summary of mean (± S.E.M) maximal core temperature drops following the bilateral injection of AVP (filled bars) or aCSF (open bars) into sites outside the meA of febrile or afebrile animals ($P > 0.05$).

Fig.7. Schematic histological sections of the rat forebrain showing injection sites that were localized to the meA (filled circles) or to sites surrounding but not impinging upon the meA (open circles) for animals in the third (upper panel) or fourth (lower panel) series of experiments. Note that histological data obtained from animals in which tissue injection was localized to the meA on at least one side of the brain are summarized in the left side of both panels. Histological data from animals receiving injections outside the meA on both sides of the brain are summarized in the right side of both panels. For clarity, injection sites localized to the meA are placed on one side of the appropriate histological sections. Abbreviations: 3v, third ventricle; ah, anterior hypothalamus; bl, basolateral amygdaloid nucleus; cc, corpus callosum; ce, central amygdaloid nucleus; hp, hippocampal area; ic, internal capsule; me, medial amygdaloid nucleus; vh, ventromedial hypothalamus.

Series III



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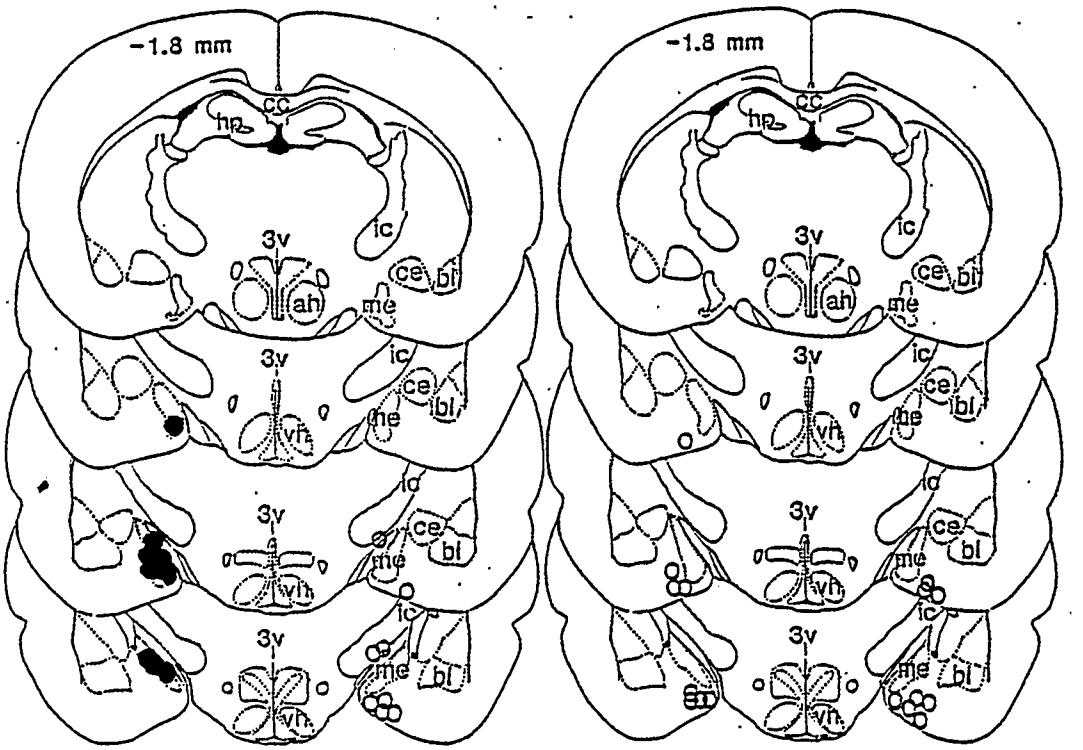


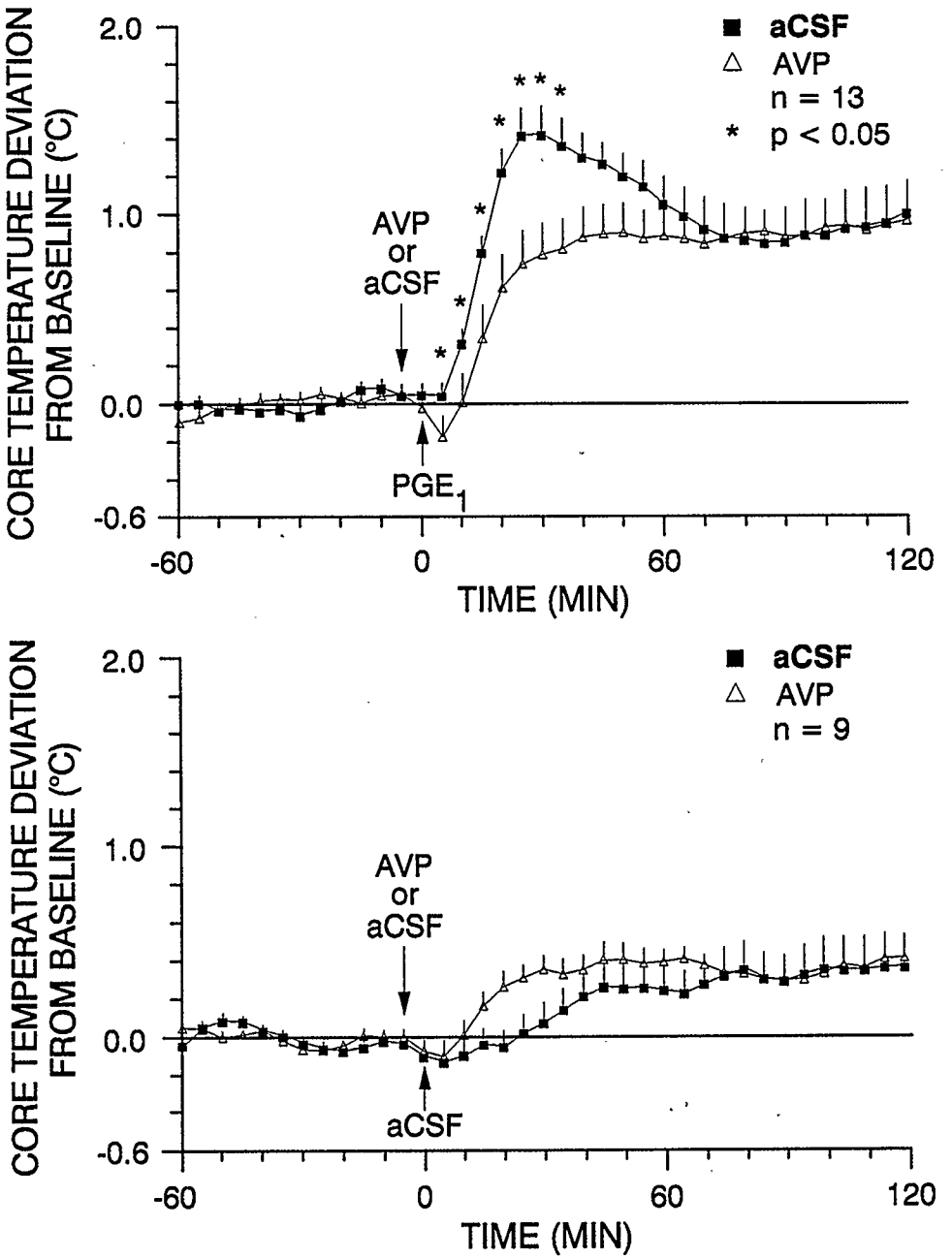


Fig. 8. Top panel: Mean temperature responses (± S.E.M) to the i.c.v. injection of PGE_1. Five minutes prior to this, either aCSF (filled squares) or AVP (open triangles) were bilaterally injected into the meA. Bottom panel: Mean (± S.E.M) temperature responses to the i.c.v. injection of aCSF. Five minutes prior to this, either aCSF (filled squares) or AVP (filled squares) were bilaterally injected into the meA (*$P < 0.05$).

average baseline core temperature of 37.2 ± 0.1°C, i.c.v. injection of PGE_1 (50 ng) with aCSF pretreatment resulted in an immediate rise in core temperature that reached an average maximum of 38.7 ± 0.1°C (or 1.5 ± 0.1°C above baseline) within 15 - 35 min. However, when the i.c.v. injection of PGE_1 was preceded by bilateral injection of AVP (40 pmol/1μl per side) into the meA, the maximal rise in core temperature from a baseline value of 37.3 ± 0.2°C was attenuated to 1.0 ± 0.2°C above baseline (n = 13; P < 0.05; Fig. 8, top panel). In addition, AVP injection into the meA did not significantly alter normothermic core temperature compared to similar injection of aCSF into the meA (n =9; P > 0.05; Fig. 8, bottom panel).

When data from animals receiving tissue injections outside the meA were analyzed separately, it was apparent that AVP injections failed to alter the hyperthermic effect of PGE_1. Thus, with AVP pretreatment, the maximum rise in core temperature in response to i.c.v. PGE_1 was 1.0 ± 0.1°C above a baseline of 37.1 ± 0.1°C, while with control aCSF pretreatment, the maximal rise in response to i.c.v. PGE_1 was also 1.0 ± 0.1°C above a baseline 37.0 ± 0.1°C (n = 5; P > 0.05; Fig. 9, top panel). In addition, AVP injected into sites outside the meA did not significantly alter normothermic core temperature compared to control injection of aCSF (n = 4; P > 0.05; Fig. 9, bottom panel).

D. DISCUSSION

The present study was carried out to determine if AVP, injected into the meA, could evoke a drop in core temperature of rats made febrile by IL-1. Further, these experiments were carried out to determine if AVP, injected into the meA, could attenuate PGE_1-induced hyperthermia in the rat. Finally, AVP effects on

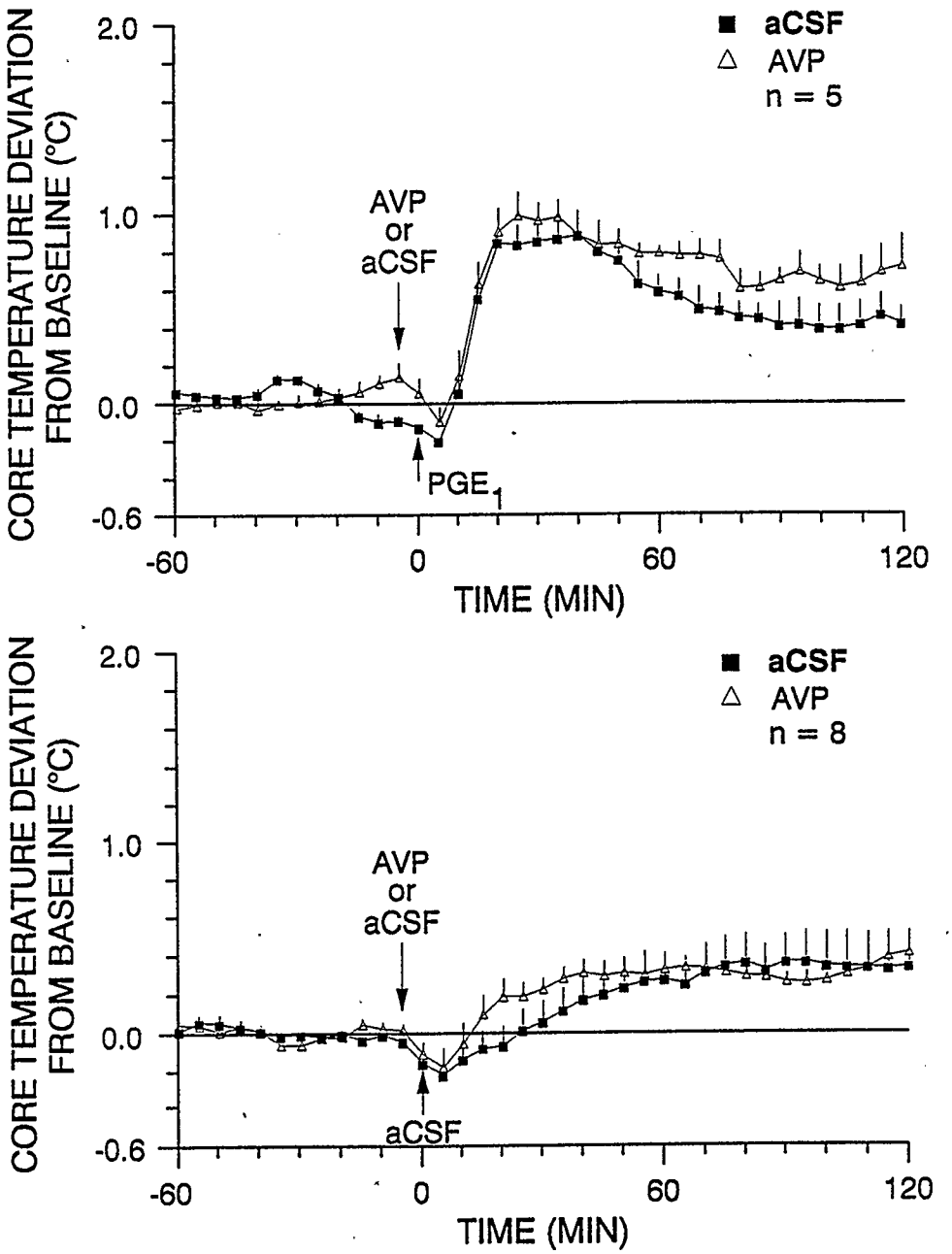


Fig. 9. Top panel: Mean temperature responses (± S.E.M) to the i.c.v. injection of PGE_1. Five minutes prior to this, either aCSF (filled squares) or AVP (open triangles) were bilaterally injected into sites outside the meA. Bottom panel: Mean (± S.E.M) temperature responses to the i.c.v. injection of aCSF. Five minutes prior to this, either aCSF (filled squares) or AVP (open triangles) were bilaterally injected into sites outside the meA ($P > 0.05$).

afebrile core temperature were assessed.

An antipyretic agent is defined as a substance which diminishes febrile body temperature while having no effect on afebrile body temperature (Barbour, 1921). The results of this study show that AVP injected into the meA attenuated both IL-1 fevers and PGE_1 hyperthermia while having no significant effect on afebrile core temperature. Thus, it is suggested that AVP is an effective antipyretic when injected into the meA of the rat. Furthermore, this antipyretic effect appears to be specific to the meA since AVP injected into sides outside the meA was not antipyretic.

The findings of this study are consistent with the hypothesis that AVP may normally be released into the meA to function as an endogenous antipyretic to limit the magnitude and duration of fevers. Support for the endogenous release of AVP into the meA comes from previous studies demonstrating an increase in AVP immunoreactivity in amygdalar nerve terminals during fever and during the development of pyrogen tolerance in pregnant and nonpregnant animals (Kasting and Martin, 1983; Zeisberger et al., 1983; Zeisberger et al., 1986; Merker et al., 1980; Zeisberger et al., 1981; Cooper et al., 1988).

As discussed in Chapter 1, it is possible that PGE_1 is a central mediator of fever and that IL-1 could evoke fevers through the elaboration and action of PGE_1 (cf. Fig. 1). Thus, one mechanism of action through which AVP injected into the meA attenuates IL-1 fevers or PGE_1 hyperthermia could involve the disruption of events elicited by the action of PGE_1 within the meA during fever development. However, this appears unlikely since PGE_1 injected into the medial portion of amygdala does not elicit hyperthermia, suggesting that prostaglandins may not directly interact with neural elements in this site in the normal genesis of fevers

(Williams et al., 1977). Alternatively, AVP might act as a neurotransmitter to modulate certain neuronal pathways that may directly or indirectly influence the ability of IL-1 or PGE_1 to evoke fevers. Consistent with this possibility are studies demonstrating the presence of V_{1a}-like AVP receptors (Freund-Mercier et al., 1988b; Dorsa et al., 1984; Lawrence et al., 1988) and vasopressinergic nerve terminals (De Vries et al., 1985; Buijs and Swaab, 1979) within the meA as well as afferent and efferent connections linking the meA with sites important in fever and antipyresis such as the POAH, VSA, PVN and BST (Disturnal et al., 1985; Mathieson et al., 1989a; Conrad and Pfaff, 1976b; Conrad and Pfaff, 1976a; Ottersen, 1980; Burnard et al., 1983; Sawchenko and Swanson, 1983; Tribollet and Dreifuss, 1981; Krettek and Price, 1978; Weller and Smith, 1982; Sofroniew, 1980; Zimmerman et al., 1984; De Vries et al., 1985). However, conclusive support for this possibility awaits further investigation.

Intracerebroventricular injection of 5 pmol of AVP in the rat has been shown to evoke larger (0.8-1.0°C) drops in febrile core temperature than those observed in this study (Kasting and Wilkinson, 1986; Poulin, personal communication). In addition, AVP has been shown to evoke antipyresis when injected into the VSA at doses as low as 0.5 pmol ($1/80^{th}$ of the dose used in this study; Naylor, 1987; Zeisberger, 1989). Taken together, these findings could suggest that the meA is not the site where AVP exerted its antipyretic effects in this study. Instead it might be argued that AVP injected into the meA may have reached the VSA to evoke antipyresis by diffusing to the VSA either directly through brain tissue, or indirectly via the cerebral ventricular system. In disagreement with this suggestion, however, AVP was not antipyretic when injected into sites outside the meA but adjacent to

the cerebral ventricles. Further, AVP was not antipyretic when injected into sites anterior to the meA that approach the VSA. Thus, it is suggested that the AVP antipyresis observed in this study is due to the site specific action of the peptide within the meA.

In conclusion, the meA appears to be a novel site where exogenously administered AVP is antipyretic. This finding is consistent with the hypothesis that AVP may function within the meA as part of an endogenous antipyretic system. Future studies will carry out a direct comparison of AVP antipyretic sensitivity between the meA and the VSA.

CHAPTER 3

ANTIPYRETIC ACTION OF VASOPRESSIN IN THE URETHANE-ANAESTHETIZED RAT

A. INTRODUCTION

The previous study demonstrated that microinjection of 40 pmol of AVP into the meA attenuates fevers evoked by i.c.v. IL-1 and PGE_1 (cf. Figs. 5 and 8). Vasopressin microinjected into the VSA at doses as low as 0.5 pmol has also been shown to attenuate fevers elicited by systemic administration of IL-1 (Naylor, 1987; Zeisberger, 1989). Thus, it is possible that AVP may be a more effective antipyretic in the VSA than in the meA. However, unequivocal support for this can only come from a direct comparison of AVP antipyretic effectiveness in the two sites using identical experimental procedures. Such studies, however, have not been carried out.

Recently, it has been demonstrated that rats under urethane-anaesthesia, though incapable of regulating body temperature, are capable of developing fevers accompanied by characteristic effector responses such as piloerection, shivering and peripheral vasoconstriction (Malkinson et al., 1988; Landgraf et al., 1990). Thus, the urethane-anaesthetized rat model could be useful for future fever research. Additionally, this model might be useful for antipyresis research, however, it is not known whether AVP is antipyretic in the anaesthetized animal.

Thus, the present experiments were undertaken to determine if AVP, exogenously administered within the VSA and the meA, could suppress PGE_1 evoked hyperthermia in the urethane-anaesthetized rat. In addition, a comparison of the antipyretic effectiveness of AVP given within the VSA versus the meA was carried out.

B. METHODS

Sixty six adult male Wistar rats weighing 275 - 375 g were anaesthetized with intraperitoneal urethane (1.5 g/kg, Sigma) to a stage III, plane 1 level as evaluated by skeletal muscle relaxation, eye and palpebral responses to stimuli, and respiratory pattern (Soma, 1971). Using the stereotaxic coordinate system of Paxinos and Watson (1982), 20-gauge stainless steel guide cannulae were positioned bilaterally over the lateral cerebral ventricles as well as the VSA or the meA so that the tips rested on dura mater. Three anchor screws were inserted into the calvarium and the cannulae were secured in place with cranioplast cement. Following surgery, colonic temperature was monitored at 5 or 10 min intervals by a thermistor probe (YSI type 701) inserted 6 cm beyond the anus. Baseline colonic temperature was maintained at 37 - 37.5°C by use of a heating pad controlled by a variable transformer. All experimental trials were carried out between 0800 - 1600 h, the time when body temperature in the conscious rat is relatively stable (Malkinson et al., 1990).

To microinject PGE_1 (Sigma), a 25-gauge stainless steel injector needle was lowered into the left lateral cerebral ventricle. The needle was connected by a length of PE-20 tubing filled with PGE_1 (30 or 40 $\mu g/\mu l$) and 5 μl of this solution was injected i.c.v. by gravitational flow over 45 seconds. Stock PGE_1 solution was prepared and stored as described previously.

To perfuse AVP (6.5 μM, Bachem) or vehicle (aCSF) within the meA or the VSA, push-pull cannulae (Fig. 10) were lowered through the guide tubes to the level of the meA or VSA. The push-push cannulae consisted of an inner, or push, cannula cut from 30-gauge stainless steel tubing that was inserted into an outer, or pull, cannula cut from 23-gauge (thin-wall) stainless steel tubing. The bevelled tip

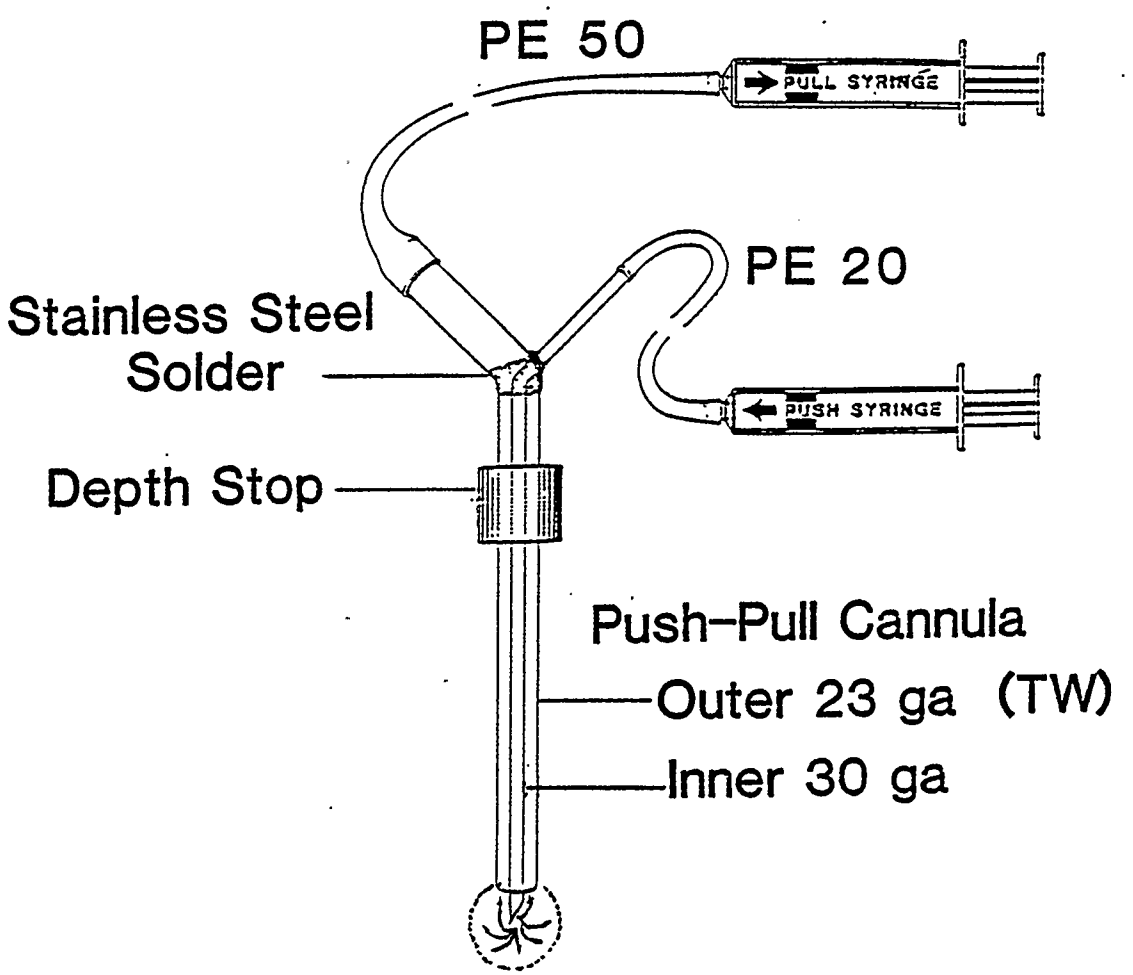


Fig. 10. Schematic diagram of the push-pull cannula perfusion system used in this study.

of the push cannula was extended 0.5 mm beyond the tip of the pull cannula. With this tip separation, it was possible to bathe a sphere of tissue approximately 1.0 mm in diameter (Myers, 1971). The push and pull cannulae were connected by PE-20 and PE-50 tubing, respectively, to gas tight 2.5 ml glass syringes mounted on a Harvard infusion/withdrawal pump that had been previously calibrated to deliver 16 μl/min. AVP stock solution was prepared and stored as described previously. Push syringes containing AVP solution were kept on ice for the duration of the experiment.

Series I

In the first series of experiments, three groups of 10 animals were used. Guide cannulae bilaterally directed toward the VSA and lateral cerebral ventricles were implanted into animals in the first 2 groups. Animals in the third group, on the other hand, had only ventricular guide cannulae implanted into them. After surgery, the animals were allowed a 30 min stabilization period. Following this stabilization period, AVP (6.5 μM; first group) or vehicle (aCSF; second group) was bilaterally push-pull perfused within the VSA for 120 min prior to an i.c.v. injection of PGE_1 (200 ng/5 μl) and for an additional 120 min after injection. For the third group of animals, a similar experimental time course was followed and PGE_1 (200 ng/5 μl) was injected i.c.v., however, no tissue perfusion was carried out. Note that on each day, one animal from each experimental group was tested and that all animals were subjected to a single experimental trial.

Series II

In a second series of experiments, a similar protocol to that outlined previously was followed, however, tissue perfusion was directed toward the meA rather than the VSA in 3 groups of 12 animals. In addition, 150 ng/5 μl PGE_1 were injected i.c.v. as opposed to 200 ng/5 μl. A lower dose of PGE_1 was used since preliminary trials indicated that the fresh PGE_1 stock solution prepared for this series of experiments was more pyrogenic than that used previously.

At the end of an experimental trial in both series of experiments, each rat was anaesthetized deeply with urethane. Perfusion sites were marked by 1 μl injections of Pelican Special black ink. After the heart was clamped, the brain was perfused with saline followed by neutral formalin by retrograde injection into the aorta. The brain was then removed and blocked in the coronal plane and sectioned at 60 μm on a sledge microtome. Each section was stained with neutral red and the injection sites were localized by light microscopy.

All temperature data were presented as means (± S.E.M) of deviations from the colonic temperature recorded at the time when PGE_1 was injected intracerebroventricularly. In this study, this time was taken to represent time 0. Temperature data were subjected to a one way Analysis of Variance followed by an unpaired Student's *t*-distribution. A fever index, defined as the area under temperature-time curve, was computed for temperature responses observed for 2 hr following PGE_1 injection and expressed as °C·hr. For all tests, statistical significance was set at $P < 0.05$.

C. RESULTS

Series I

From an average colonic temperature of 37.4 ± 0.1°C, i.c.v. injection of PGE_1 (200 ng) with concurrent perfusion of aCSF within the VSA evoked an immediate rise in colonic temperature that reached a maximum of 38.7 ± 0.2°C (or 1.3 ± 0.2°C above baseline) within 15 - 35 min. However, as shown in Fig. 11, when the i.c.v. injection of PGE_1 was made during AVP (6.5 μM) perfusion within the VSA, the maximal rise in colonic temperature from a baseline value of 37.4 ± 0.2°C was attenuated to 0.3 ± 0.3°C above baseline (n = 10; P < 0.01). In addition, AVP perfusion prior to PGE_1 injection did not significantly alter normothermic core temperature compared to similar perfusion of aCSF (P > 0.05; Fig. 11, top panel). As illustrated in Fig. 12, fever indices of the PGE_1 hyperthermia were significantly attenuated during AVP perfusion within the VSA compared to control aCSF perfusion (aCSF = 1.8 ± 0.2°C·hr; AVP = 0.2 ± 0.3°C·hr; n = 10; P < 0.01). The PGE_1 hyperthermia elicited in animals during aCSF perfusion within the VSA were not significantly different from those elicited in animals that received no tissue perfusion (n = 10; P > 0.05; Fig. 11, bottom panel).

Histological examination of tissue perfusion sites of the 10 animals used in the first experimental series shows that perfusion was localized to the VSA and surrounding areas (Fig. 13). AVP effectively attenuated PGE_1 hyperthermia in all perfusion sites. Thus, animals were not divided on the basis of perfusion sites in this series of experiments.

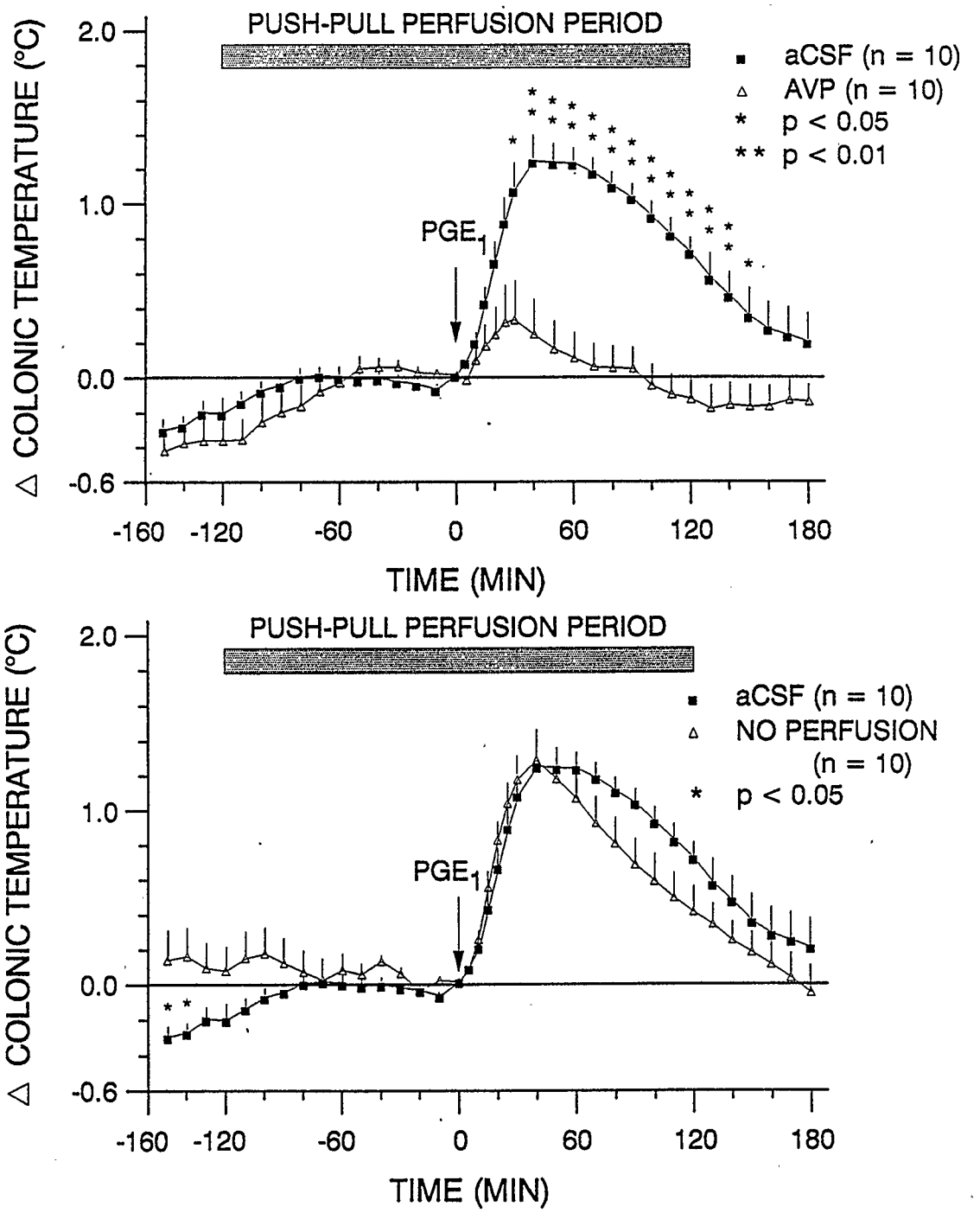


Fig. 11. Top panel: Mean (± S.E.M.) changes in colonic temperature in response to the i.c.v. injection of PGE_1 during perfusion of aCSF (filled squares) or AVP (open triangles) within the VSA. Bottom panel: Mean (± S.E.M.) changes in colonic temperature in response to i.c.v. injection of PGE_1 during aCSF perfusion within the VSA (filled squares) or in the absence of tissue perfusion (open triangles) (*$P < 0.05$; **$P < 0.01$).

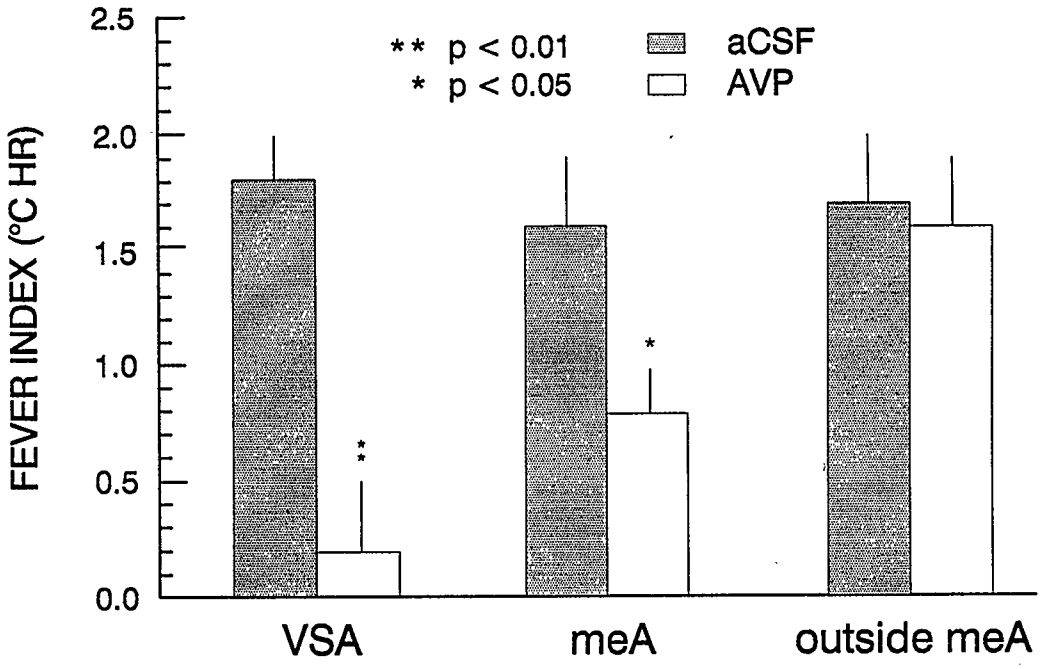


Fig. 12. Summary of mean (± S.E.M.) fever indices of PGE_1 hyperthermia elicited during perfusion of aCSF (filled bars) or AVP (open bars) within the VSA (left), meA (middle) or sites outside the meA (right). Note that fever indices were calculated for 2 hours following the i.c.v. injection of PGE_1. (VSA perfusion, n = 10 per group; meA perfusion, n = 7 per group; perfusion outside the meA, n = 5 per group; $*P < 0.05$; $**P < 0.01$).

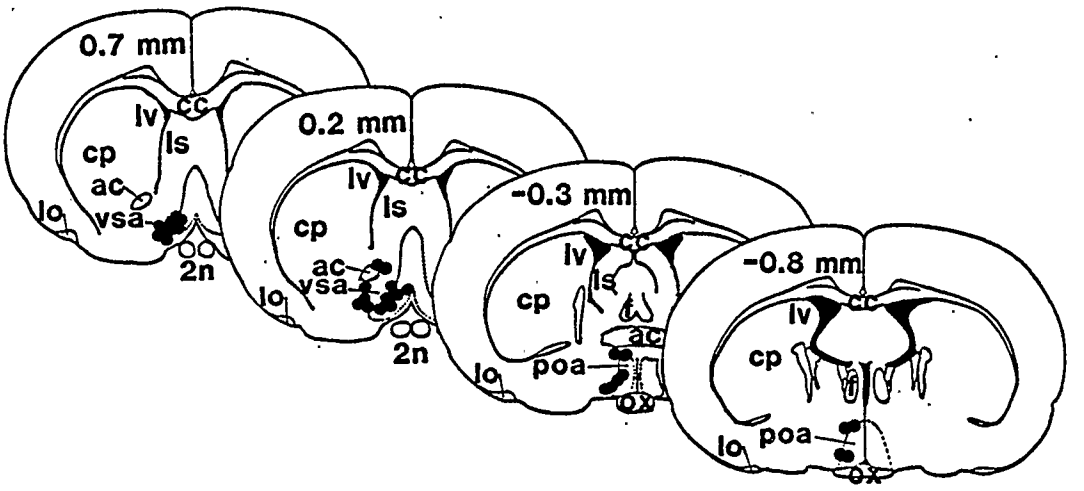


Fig. 13. Histological representation of coronal sections of the rat forebrain taken at 60 μm indicating sites at which AVP was effective (filled circles) in attenuating PGE_1 hyperthermia in the first series of experiments. Abbreviations: 2n, optic nerve; ac, anterior commissure; cc, corpus callosum; cp, caudate putamen; f, fornix; lv, lateral ventricle; lo, lateral olfactory tract; ls, lateral septum; ox, optic chiasm; poa, preoptic area; vsa, ventral septal area.

Series II

Histological examination of tissue perfusion sites of the 12 animals used in the second series of experiments showed that push-pull perfusion was localized to the meA and to adjacent areas (Fig 14). Animals were therefore separated into two groups for data analysis, those receiving tissue perfusion within the meA on at least one side of the brain, and those receiving tissue perfusion in sites surrounding but not impinging upon the meA on both sides of the brain.

When PGE_1 (150 ng) was injected i.c.v. during aCSF perfusion within the meA, colonic temperature increased from 37.0 ± 0.2°C to a peak of 1.4 ± 0.3°C above baseline within 15 - 35 min. However, this temperature rise was limited to 0.7 ± 0.2°C above baseline values of 37.1 ± 0.2°C when AVP (6.5 μM) was perfused within the meA (n = 7; P < 0.05; Fig. 15, top panel). In addition, AVP perfusion prior to PGE_1 injection did not significantly alter normothermic core temperature compared to similar perfusion of aCSF (P > 0.05; Fig. 15, top panel). As shown in Fig. 12, fever indices of the PGE_1 hyperthermia were significantly reduced by AVP perfusion within the meA relative to control perfusion of aCSF (aCSF = 1.6 ± 0.3°C·hr; AVP = 0.8 ± 0.2°C·hr; n = 7; P < 0.05) Animals that received no tissue perfusion responded to i.c.v. PGE_1 with hyperthermia that were not significantly different from those elicited in animals receiving aCSF perfusion within the meA (n = 7; P > 0.05; Fig. 15, bottom panel). A comparison of colonic temperature responses to i.c.v. PGE_1 during AVP perfusion within the VSA versus the meA showed that AVP antipyresis was more pronounced in the VSA. (P < 0.01; Figs. 11 and 15).

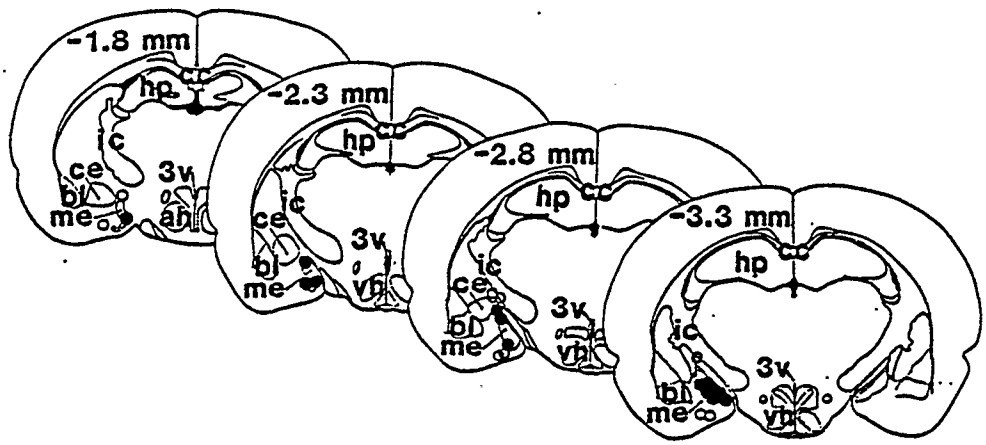


Fig. 14. Histological representation of coronal sections of the rat forebrain taken at 60 μm indicating sites at which AVP was effective (filled circles) or ineffective (open circles) in attenuating PGE_1 hyperthermia in the second series of experiments. Abbreviations: 3v, third ventricle; ah, anterior hypothalamus; bl, basolateral amygdaloid nucleus; cc, corpus callosum; ce, central amygdaloid nucleus; hp, hippocampal area; ic, internal capsule; me, medial amygdaloid nucleus; vh, ventromedial hypothalamus.

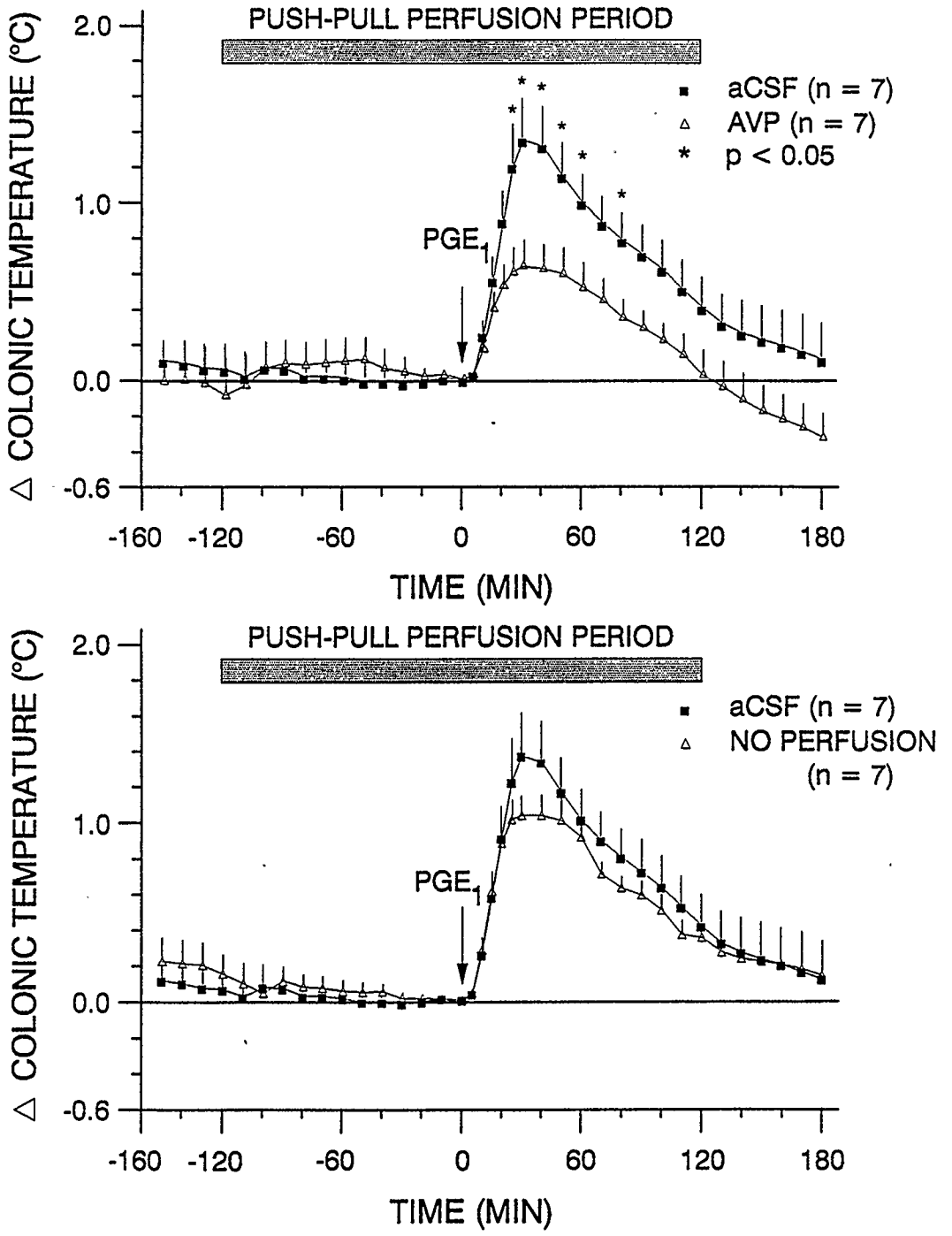


Fig. 15. Top panel: Mean (± S.E.M.) changes in colonic temperature in response to the i.c.v. injection of PGE_1 during aCSF (filled squares) or AVP (open triangles) perfusion within the meA. Bottom panel: Mean (± S.E.M.) changes in colonic temperature in response to i.c.v. injection of PGE_1 during aCSF perfusion within the meA (filled squares) or in the absence of tissue perfusion (open triangles) (*$P < 0.05$).

When data from animals receiving tissue perfusion of sites outside the meA were analyzed separately, it was apparent that AVP perfusion failed to significantly alter the hyperthermic effect of PGE_1. Thus, during AVP perfusion, the maximum rise in colonic temperature in response to i.c.v. PGE_1 was 1.5 ± 0.3°C above baseline, whereas during aCSF perfusion, the rise was 1.2 ± 0.2°C above baseline (n = 5; P > 0.05; Fig. 16, top panel). In addition, fever indices of the PGE_1 hyperthermia were not significantly reduced by AVP perfusion of sites outside the meA relative to control aCSF perfusion (aCSF = 1.7 ± 0.3°C·hr; AVP = 1.6 ± 0.3°C·hr; P > 0.05; Fig. 12, right). The PGE_1 hyperthermia elicited in animals during aCSF perfusion of sites surrounding the meA were not significantly different from those elicited in animals that received no perfusion (n = 5; P > 0.05; Fig. 16, bottom panel).

D. DISCUSSION

The present study was carried out to determine if AVP, perfused within the VSA and meA, could suppress PGE_1-induced hyperthermia in the urethane-anaesthetized rat. Further, a comparison of the antipyretic effectiveness of AVP perfused within the meA versus the VSA was carried out.

An antipyretic agent is defined as a substance which diminishes febrile body temperature while having no effect on afebrile body temperature (Barbour, 1921). The results of first series of experiments show that AVP perfused within the VSA suppresses PGE_1 hyperthermia while having no significant effect on normothermic colonic temperature. Thus, it is suggested that AVP is an effective antipyretic when perfused within the VSA of the urethane-anaesthetized rat. These findings are

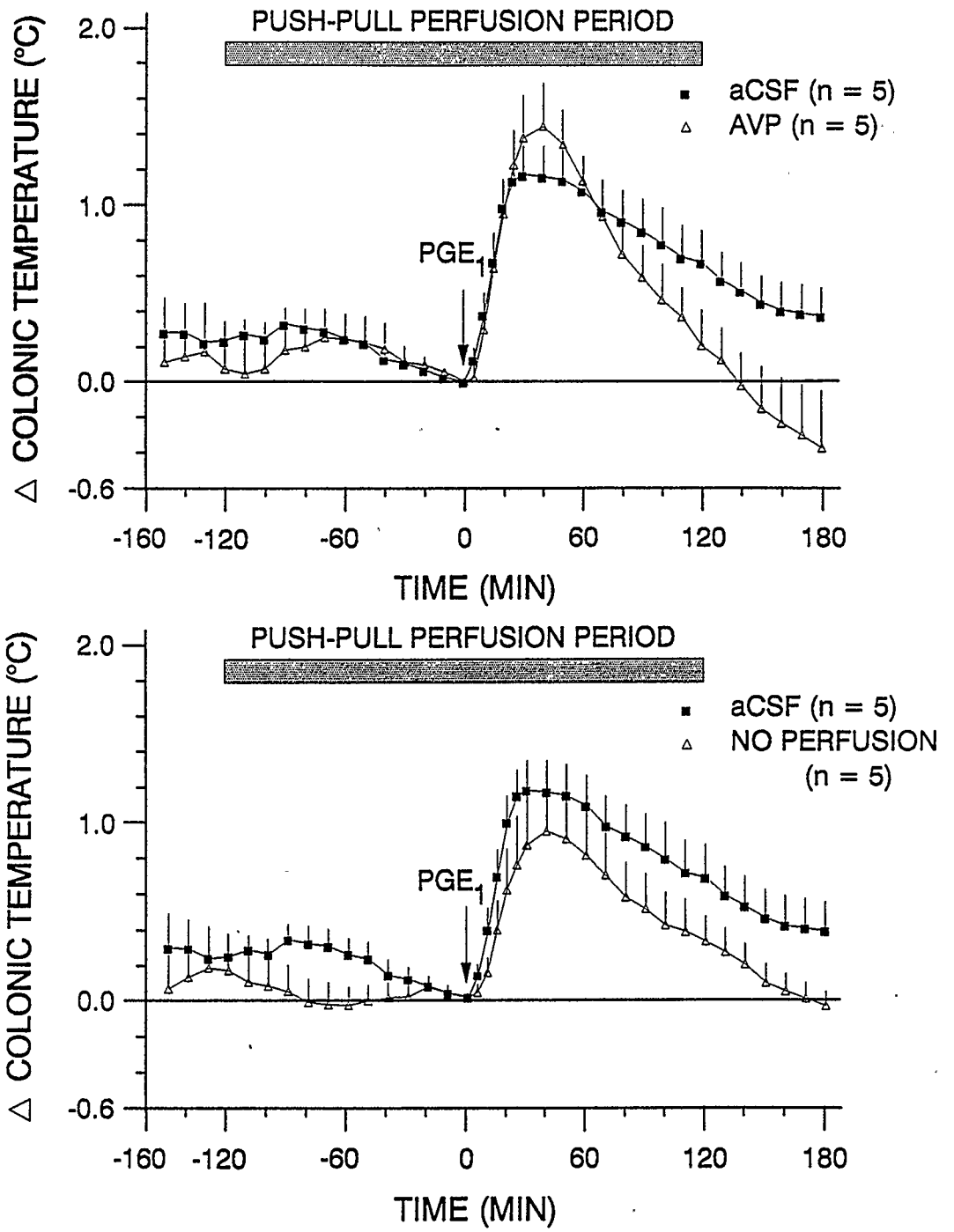


Fig. 16. Top panel: Mean (± S.E.M.) changes in colonic temperature in response to the i.c.v. injection of PGE_1 during aCSF (filled squares) or AVP (open triangles) perfusion of sites outside the meA. Bottom panel: Changes (± S.E.M.) in colonic temperature in response to i.c.v. injection of PGE_1 during aCSF perfusion of sites outside the meA (filled squares) or in the absence of tissue perfusion (open triangles) ($P > 0.05$).

consistent with earlier work using conscious rats and other species which demonstrate AVP-induced antipyresis in the VSA using prostaglandins and a variety of other pyrogens (Naylor et al., 1985b; Kasting et al., 1979a). It was also observed that push-pull perfusion of aCSF had no significant effect on PGE_1 hyperthermia, indicating that this perfusion technique did not introduce a systematic error into temperature data obtained in this study. Consequently, these findings provide further support for the utility of the urethane-anaesthetized rat model for fever and antipyresis research.

The results of the second series of experiments show that AVP perfused within the amygdala is antipyretic since this peptide attenuated PGE_1 hyperthermia while not significantly affecting afebrile colonic temperature. Further, this antipyretic effect appears to be site specific to the meA since AVP perfusion of surrounding areas were ineffective in attenuating PGE_1-evoked hyperthermia. These findings are consistent with findings presented earlier in this thesis and with the hypothesis that AVP may function within the meA as an endogenous antipyretic to limit the magnitude and duration of fevers. Furthermore, these findings also support the utility of the urethane-anaesthetized rat model for future investigations.

It was observed that AVP perfused within the meA suppresses PGE_1 hyperthermia less effectively than AVP perfused within the VSA at the same concentration and that AVP sensitive sites appear to be restricted to a smaller area surrounding the meA than surrounding the VSA. Though the physiological significance of this remains to be determined, these differences may be due to several factors. For example, is possible that urethane anaesthesia may have altered neuronal properties in amygdalar neurons in a manner that attenuates AVP effects

in this site. This possibility, is unlikely since AVP antipyresis also appears to be less pronounced in the meA compared to the VSA in conscious rats (Federico and Veale, 1990). Among other possibilities, if AVP-induced antipyresis in the meA is assumed to be receptor mediated in a similar manner to that within the VSA (Cooper et al., 1987; Naylor et al., 1987), then perhaps differences in receptor concentration, distribution area or AVP binding properties between the meA and VSA may account for the varying antipyretic efficacy of AVP. However, examining this possibility awaits quantitative studies of AVP receptor properties in both the meA and VSA.

In conclusion, these data support and strengthen the hypothesis that AVP might function within the meA as an endogenous antipyretic. In addition, the meA appears to be less sensitive to AVP antipyretic action than the VSA. Future studies will now be directed at characterizing the central receptor mediating AVP-induced antipyresis in the meA.

CHAPTER 4

CHARACTERIZATION OF THE CENTRAL RECEPTOR MEDIATING THE ANTIPYRETIC ACTION OF VASOPRESSIN IN THE MEDIAL AMYGDALOID NUCLEUS

A. INTRODUCTION

To further support a functional role for AVP in the meA in endogenous antipyresis, it is necessary to demonstrate AVP release into this site during fever. Kasting and Martin (1983) have shown by radioimmunoassay that AVP levels in the amygdala decrease during endotoxin fever in rats and these changes were suggested to indicate increased AVP release into this site. Other studies have shown increased immunoreactivity for AVP in amygdalar nerve terminals and paraventricular soma during fever in the nonpregnant guinea-pig (Zeisberger et al., 1983; Zeisberger et al., 1986), changes which were suggested to indicate activation of ascending vasopressinergic projections to the amygdala. These changes appeared to be similar to those observed when fever is absent at term (Merker et al., 1980; Zeisberger et al., 1981) as well as those observed during the development of pyrogen tolerance (Cooper et al., 1988).

Currently, the nature of the central receptor meditating AVP antipyretic action in the meA is not known. To date, three classes of vasopressin receptors have been identified on the basis of ligand sensitivities and second messenger system activation, namely, V_{1a} (vasopressor), V_{1b} (adenohypophyseal), and V_2(antidiuretic) (Mitchell et al., 1979; Brown et al., 1963; Kirk et al., 1979; Jard et al., 1986). In the CNS there is evidence that a V_{1a}-like receptor mediates AVP effects on hippocampal neuronal firing (Tiberiis et al., 1983), behavioral convulsive activity (Naylor et al., 1985a; Burnard et al., 1986), cardiovascular regulation (Matsuguchi et al., 1982) and

septal antipyresis (Cooper et al., 1987; Naylor et al., 1988). Further, AVP effects on electrical activity in the guinea-pig supraoptic nucleus have been attributed to V_2 receptor activation (Abe et al., 1983). A functional role for AVP receptors in the amygdala, however, remains to be established.

Experiments were therefore undertaken to characterize the central receptor mediating AVP-induced antipyresis in the meA and to determine whether the action of endogenously released AVP is necessary in the meA to modulate febrile body temperature. This was achieved by using relatively specific antagonists of the V_{1a} [1-(ß-mercapto-ß,ß-cyclopentamethylenepropionic acid), 2-(O-methyl)tyrosine arginine vasopressin; $d(CH_2)_5$Tyr(Me)AVP; Kruszynski et al., 1980] or V_2 [1-(ß-mercapto-ß,ß-cyclopentamethylenepropionic acid), 2-D-Valine, 4-valine arginine vasopressin; $d(CH_2)_5$-D-ValVAVP; Manning et al., 1984] subtype of peripheral vasopressin receptors, or by using a relatively specific agonist of the V_2 subtype of vasopressin receptor (1-desamino-8-D arginine vasopressin; DDAVP; Sawyer et al., 1974). These AVP derivatives were injected into the meA in order to assess their effects on AVP-induced antipyresis as well as PGE_1-evoked hyperthermia in the conscious rat.

B. METHODS

Twenty nine male Sprague-Dawley rats initially weighing 250-300 g were used. The animals were housed in a colony room maintained at 22 ± 1°C on a 12 h light-dark cycle with continuous access to food and water.

Under sodium pentobarbitol anesthesia (Somnotol; 65 mg/kg i.p.), 23-gauge (thin-wall) stainless steel guide cannulae were bilaterally implanted into each rat using stereotaxic procedures (Paxinos and Watson, 1982) so that the tips remained

5 mm above the intended site of injection into the meA. In addition, 20-gauge stainless steel guide cannulae were similarly positioned over the lateral cerebral ventricles so that the tips rested on dura mater. Three stainless steel anchor screws were inserted into the calvarium and the cannulae were secured in place with cranioplast cement. Stylets were inserted to occlude the cannulae when they were not in use. Paraffin-coated temperature-sensitive radiotransmitters (Mini Mitter Inc.) were intraperitoneally implanted into the animals for the future remote monitoring of core temperature. Following surgery, the animals were allowed a 10 day recovery period.

All animals used in this study were subjected to 3 or 4 experimental trials, the order of which was randomized and separated by at least 5 days. All experimental trials were carried out between 0800 - 1600 h, the time when body temperature in the rat is relatively stable (Malkinson et al., 1990). On each experimental test day, rats were placed individually into a plastic cage without restraint and allowed a 1 h settling period. Following this settling period, baseline recordings of core temperature were made for 1 h prior to the i.c.v. injection of PGE_1 or aCSF. Core temperature was monitored by antennae designed to receive the transmission of the intraperitoneally implanted temperature-sensitive radiotransmitters. Core temperature was recorded on-line (Dataquest III, Data Sciences Inc.) at 5 min intervals using a Heath AT computer system.

To microinject PGE_1 (50 ng; Sigma), a sterile 25-gauge stainless steel injector needle was lowered into the left lateral cerebral ventricle. The needle was connected by a length of PE-20 tubing filled with PGE_1 (10 $\mu g/\mu l$) and 5 μl of this solution was injected i.c.v. by gravitational flow over 45 seconds. Stock PGE_1

solution was prepared and stored as described previously.

All experimental animals also received control injections of aCSF (1μl per side), that were bilaterally directed toward the meA over a 1 min period. To achieve this, sterile 27-gauge injector cannulae were lowered through the guide cannulae to the level of the meA. The injector cannulae were connected by a length of PE-20 tubing to gas tight 10 μl glass syringes mounted on a Harvard infusion pump that had been previously calibrated to deliver 1 μl/min. Depending on the experimental series, animals received similar bilateral injections of AVP (40 pmol in 1μl; Bachem), DDAVP (40 pmol in 1μl; Bachem), d$(CH_2)_5$Tyr(Me)AVP (400 pmol in 1μl; Bachem) or d$(CH_2)_5$-D-ValVAVP (400 pmol in 1μl; Dr. M. Manning). These solutions were prepared immediately prior to each experiment from separate stock solutions (10^{-3} M in 0.025 M acetic acid) stored at 4°C.

Series I and II

In the first series of experiments, 11 animals were subjected to three experimental trials each. Hyperthermic responses were evoked by injecting PGE_1 (50ng/5μl) intracerebroventricularly. Fifteen min prior to this, either d$(CH_2)_5$Tyr(Me)AVP (400 pmol/1μl per side) or aCSF(1μl per side) were injected bilaterally into the meA region. As a control, aCSF (5μl) was injected i.c.v. and 15 min prior to this, d$(CH_2)_5$Tyr(Me)AVP (400 pmol/1μl per side) was injected bilaterally into the meA area.

In the second series of experiments, 10 animals were surgically prepared for experimentation. However, headplates became dislodged in 2 of the animals, thus only 8 animals were subjected to three experimental trials each. Hyperthermic

responses were evoked by injecting PGE_1 (50ng/5μl) intracerebroventricularly. Fifteen min prior to this, either d$(CH_2)_5$-D-ValVAVP (400 pmol/1μl per side) or aCSF(1μl per side) were injected bilaterally into the meA region. As a control, aCSF (5μl) was injected i.c.v. and 15 min prior to this, d$(CH_2)_5$-D-ValVAVP (400 pmol/1μl per side) was injected bilaterally into meA area.

Series III

In the third experimental series, 8 animals were subjected to four experimental trials. In all four experimental trials, hyperthermia were evoked by i.c.v. injection of PGE_1 (50ng/5μl). At 5 and 15 min prior to PGE_1 administration, respectfully, animals received bilateral injections into the meA of the following: aCSF (1μl per side) and aCSF (1μl per side), AVP (40 pmol/1μl per side) and aCSF (1μl per side), AVP (40 pmol/1μl per side) and d$(CH_2)_5$Tyr(Me)AVP (400 pmol/1μl per side), or DDAVP (40 pmol/1μl per side) and aCSF (1μl per side).

Upon completion of all experiments, each rat was anaesthetized deeply with sodium pentobarbitol. Tissue injection sites were marked by 1 μl injections of Pelican Special black ink. The brain was perfused with saline followed by neutral formalin by retrograde injection into the aorta. The brain was then removed and blocked in the coronal plane and sectioned at 60 μm on a sledge microtome. Each section was stained with neutral red and tissue injection sites were localized by light microscopy.

Animals used in this study were separated into two groups for data analysis, namely, those receiving tissue injections into the meA on at least one side of the brain, and those receiving tissue injection into sites surrounding but not impinging

upon the meA on both sides of the brain. Temperature data were presented as deviations from the mean baseline core temperature recorded for 45 min prior to tissue injection at time - 15 min. In this study, the time at which PGE_1 was administered was taken to represent time 0. All data were subjected to a one way Analysis of Variance followed by Newman Keuls post-hoc comparisons. For all tests, statistical significance was set at $P < 0.05$.

C. RESULTS

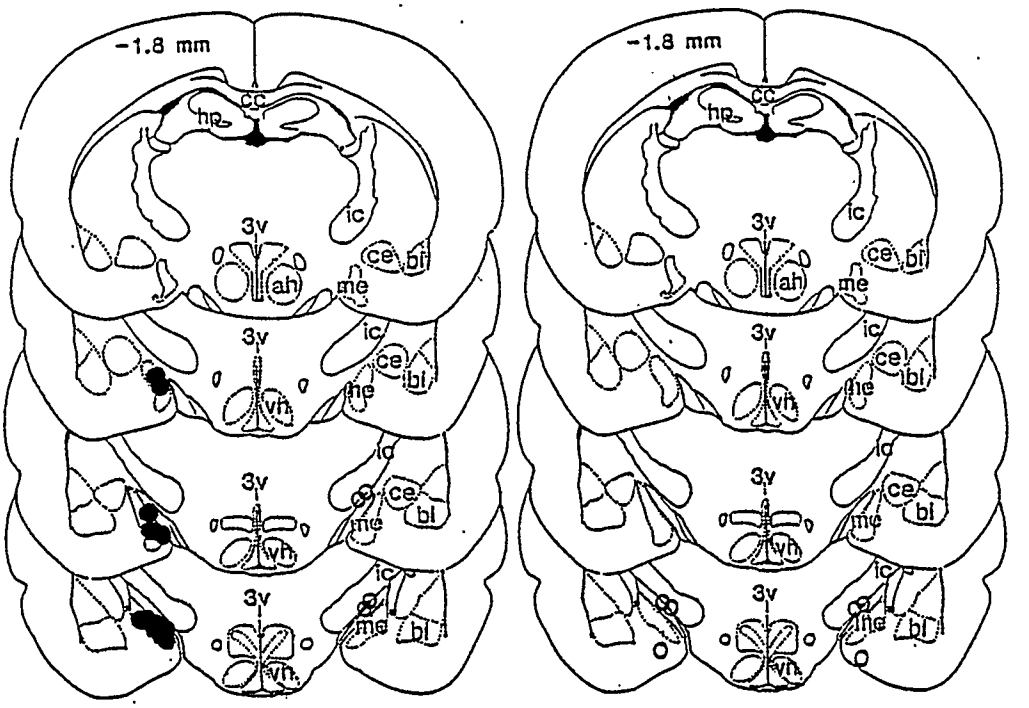
Series I and II

Histological examination of tissue injection sites of the 19 animals used in the first two experimental series showed that tissue injections were localized to the meA and adjacent areas (Fig 17). As previously mentioned, animals were separated on the basis of whether or not tissue injection was localized to the meA on at least one side of the brain.

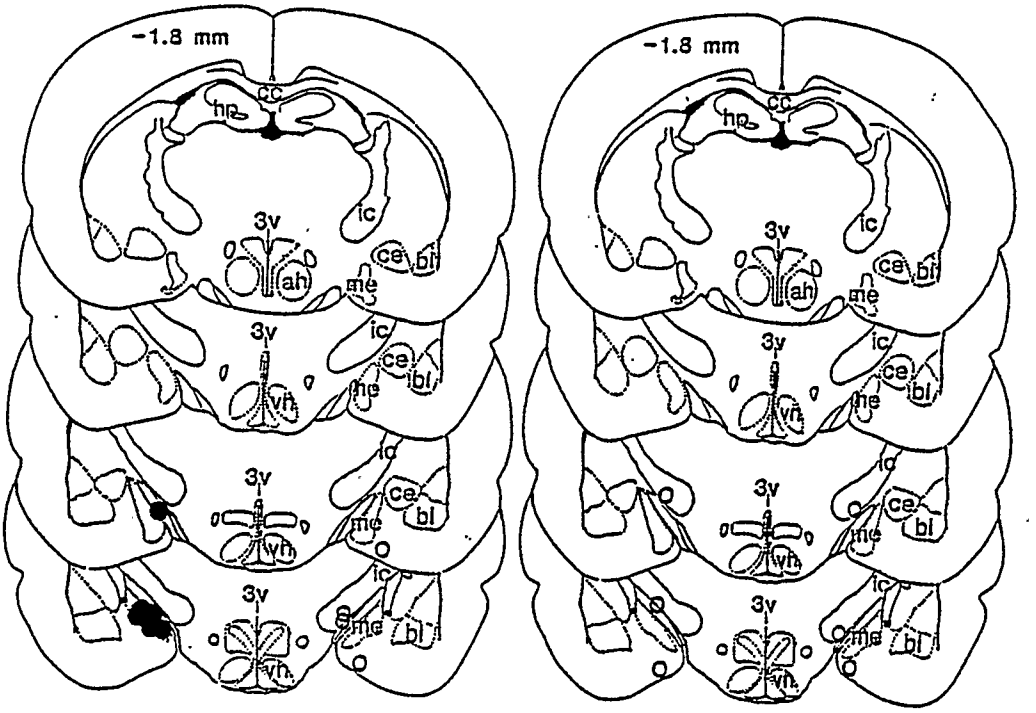
Fig. 18 illustrates results obtained from animals in the first two experimental series in which tissue injections were localized to the meA. From an average baseline core temperature of 37.3 ± 0.2°C, i.c.v. injection of PGE_1 (50 ng) with aCSF pretreatment resulted in an immediate rise in core temperature that reached an average peak of 38.5 ± 0.1°C (or 1.2 ± 0.1°C above baseline) within 15 - 40 min. When the i.c.v. injection of PGE_1 was preceded by bilateral injection of $d(CH_2)_5Tyr(Me)AVP$ (400 pmol) into the meA, the hyperthermia elicited from baseline values of 37.3 ± 0.1°C were not significantly different from aCSF controls for the first 120 min ($d(CH_2)_5Tyr(Me)AVP$: peak = 1.3 ± 0.1°C; $n = 8$; $P > 0.05$; Fig 18, top panel). After this, however, pretreatment with V_{1a} antagonist elicited a

Fig. 17. Schematic histological sections of the rat forebrain showing injection sites that were localized to the meA (filled circles) or to sites surrounding but not impinging upon the meA (open circles) for animals in the first (upper panel) or second (lower panel) series of experiments. Note that histological data obtained from animals in which tissue injection was localized to the meA on at least one side of the brain are summarized in the left side of both panels. Histological data from animals receiving injections outside the meA on both sides of the brain are summarized in the right side of both panels. For clarity, injection sites localized to the meA are placed on one side of the appropriate histological sections. Abbreviations: 3v, third ventricle; ah, anterior hypothalamus; bl, basolateral amygdaloid nucleus; cc, corpus callosum; ce, central amygdaloid nucleus; hp, hippocampal area; ic, internal capsule; me, medial amygdaloid nucleus; vh, ventromedial hypothalamus.

Series I



Series II



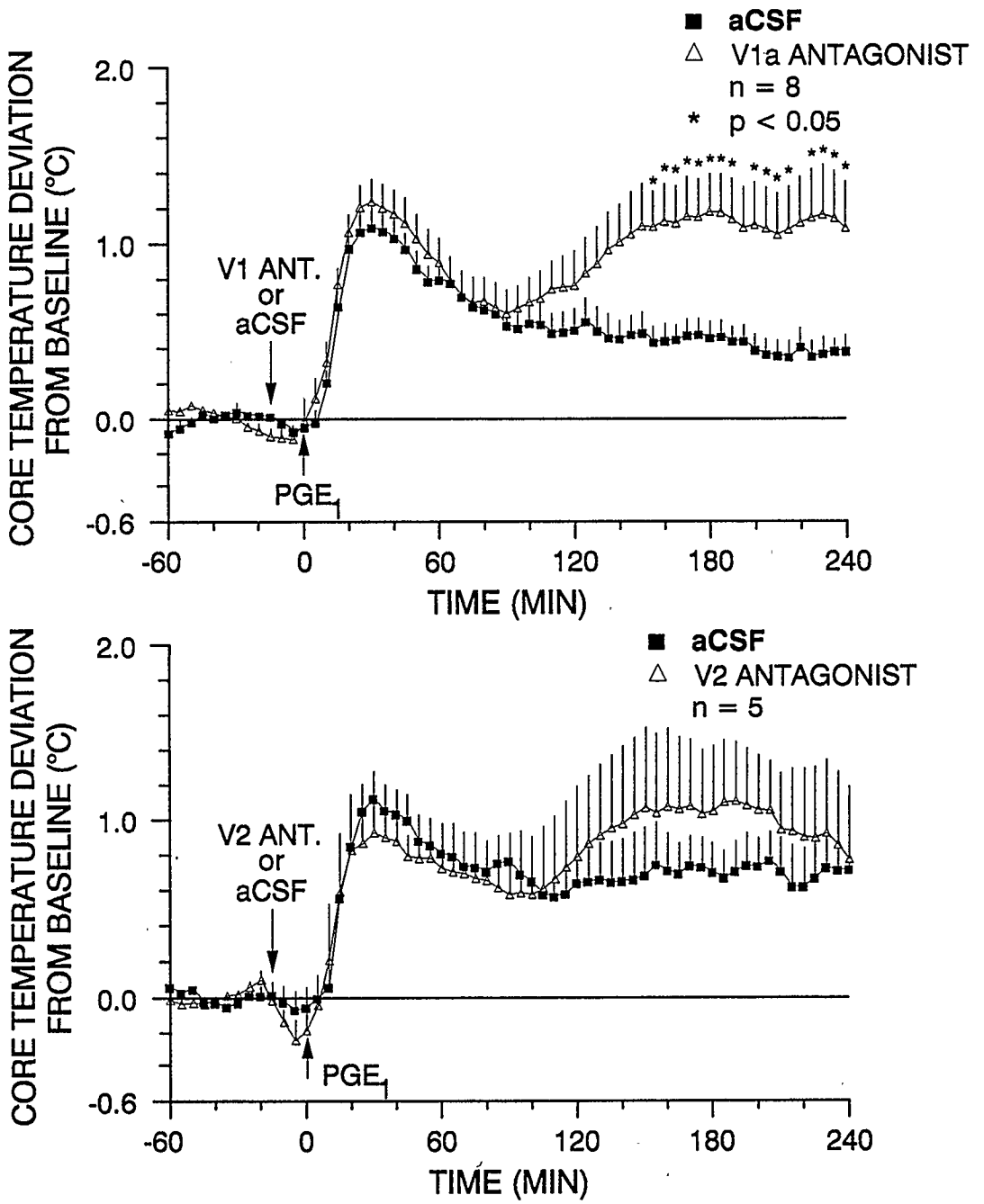


Fig. 18. Top panel: Mean (± S.E.M) temperature responses to the i.c.v. injection of PGE_1. Fifteen minutes prior to this, either aCSF (filled squares) or $d(CH_2)_5Tyr(Me)AVP$ (V_{1a} antagonist; open triangles) were bilaterally injected into the meA. Bottom panel: Mean (± S.E.M) temperature responses to the i.c.v. injection of PGE_1. Fifteen minutes prior to this, either aCSF (filled squares) or $d(CH_2)_5$-D-ValVAVP (V_2 antagonist; open triangles) were bilaterally injected into the meA (*$P < 0.05$).

secondary rise in hyperthermic core temperature that became significantly different from aCSF controls ($P < 0.05$; Fig. 18, top panel). When PGE_1 administration was preceded by $d(CH_2)_5$-D-ValVAVP injection into the meA, hyperthermia were evoked that were not significantly different from aCSF controls (aCSF: baseline = 37.3 ± 0.1°C; peak = 1.1 ± 0.2°C. $d(CH_2)_5$-D-ValVAVP: baseline = 37.4 ± 0.2°C; peak = 1.1 ± 0.3°C. $n = 5$; $P > 0.05$; Fig. 18, bottom panel).

When data from animals receiving tissue injections outside the meA were analyzed separately, it was apparent that $d(CH_2)_5$Tyr(Me)AVP or $d(CH_2)_5$-D-ValVAVP pretreatment failed to alter the hyperthermic effect of PGE_1. Thus, with $d(CH_2)_5$Tyr(Me)AVP pretreatment, the maximum rise in core temperature in response to i.c.v. PGE_1 was 1.3 ± 0.5°C above a baseline of 37.7 ± 0.2°C, while with aCSF pretreatment, the maximal rise in core temperature in response to i.c.v. PGE_1 was 1.1 ± 0.4°C above a baseline 37.6 ± 0.3°C ($n = 3$; $P > 0.05$; Fig. 19, top panel). Further, with $d(CH_2)_5$-D-ValVAVP pretreatment, the maximum rise in core temperature in response to i.c.v. PGE_1 was 1.4 ± 0.1°C above a baseline of 36.5 ± 0.5°C, while with aCSF pretreatment, the maximal rise in core temperature in response to i.c.v. PGE_1 was 1.2 ± 0.2°C above a baseline 37.0 ± 0.0°C ($P > 0.05$; Fig. 19, bottom panel). In addition, $d(CH_2)_5$Tyr(Me)AVP or $d(CH_2)_5$-D-ValVAVP injection into the meA or outside the meA did not evoke profound changes in normothermic core temperature ($P > 0.05$; Fig. 20).

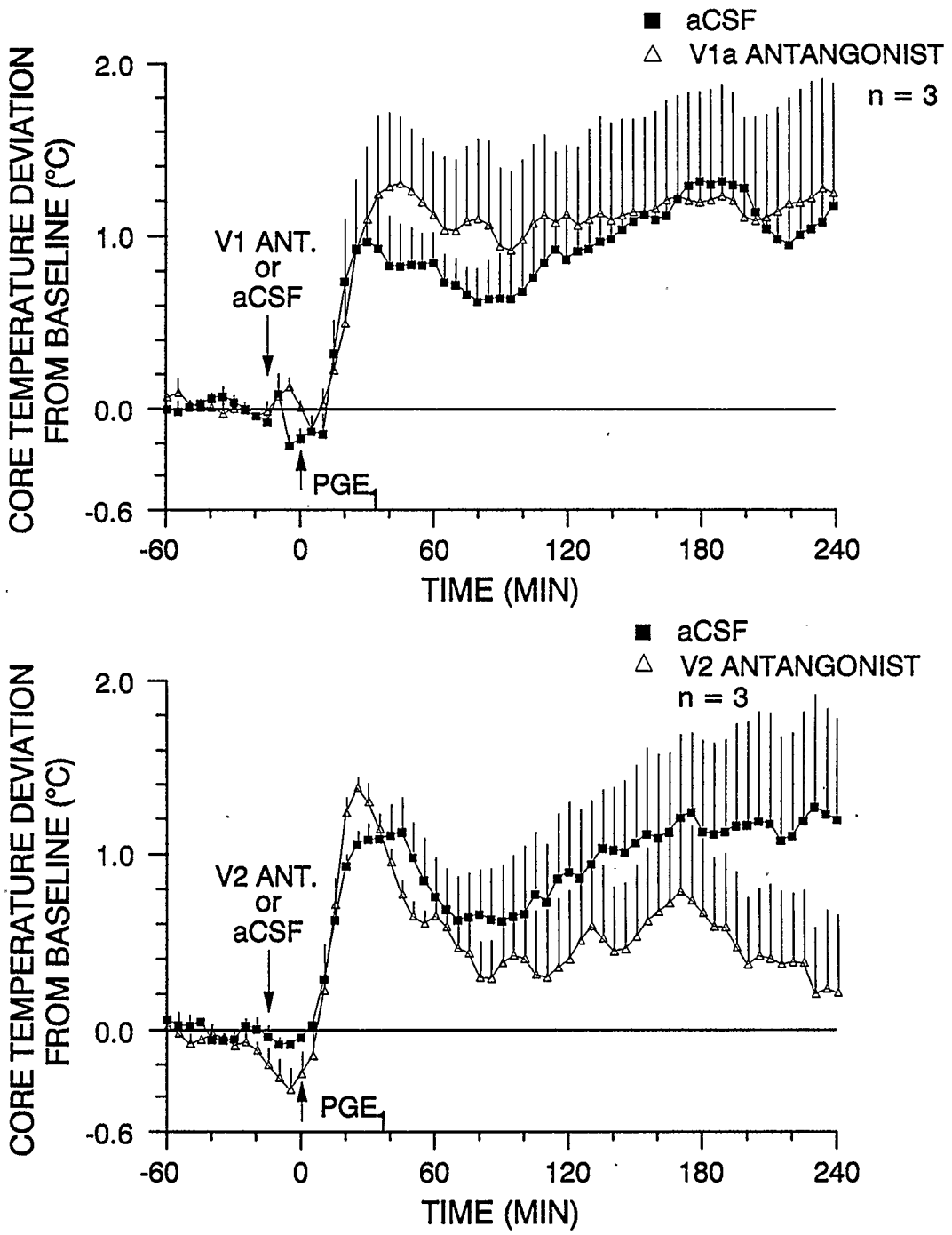


Fig. 19. Top panel: Mean (± S.E.M) temperature responses to the i.c.v. injection of PGE_1. Fifteen minutes prior to this, either aCSF (filled squares) or $d(CH_2)_5$Tyr(Me)AVP (V_{1a} antagonist; open triangles) were bilaterally injected into sites outside the meA. Bottom panel: Mean (± S.E.M) temperature responses to the i.c.v. injection of PGE_1. Fifteen minutes prior to this, either aCSF (filled squares) or $d(CH_2)_5$-D-ValVAVP (V_2 antagonist; open triangles) were bilaterally

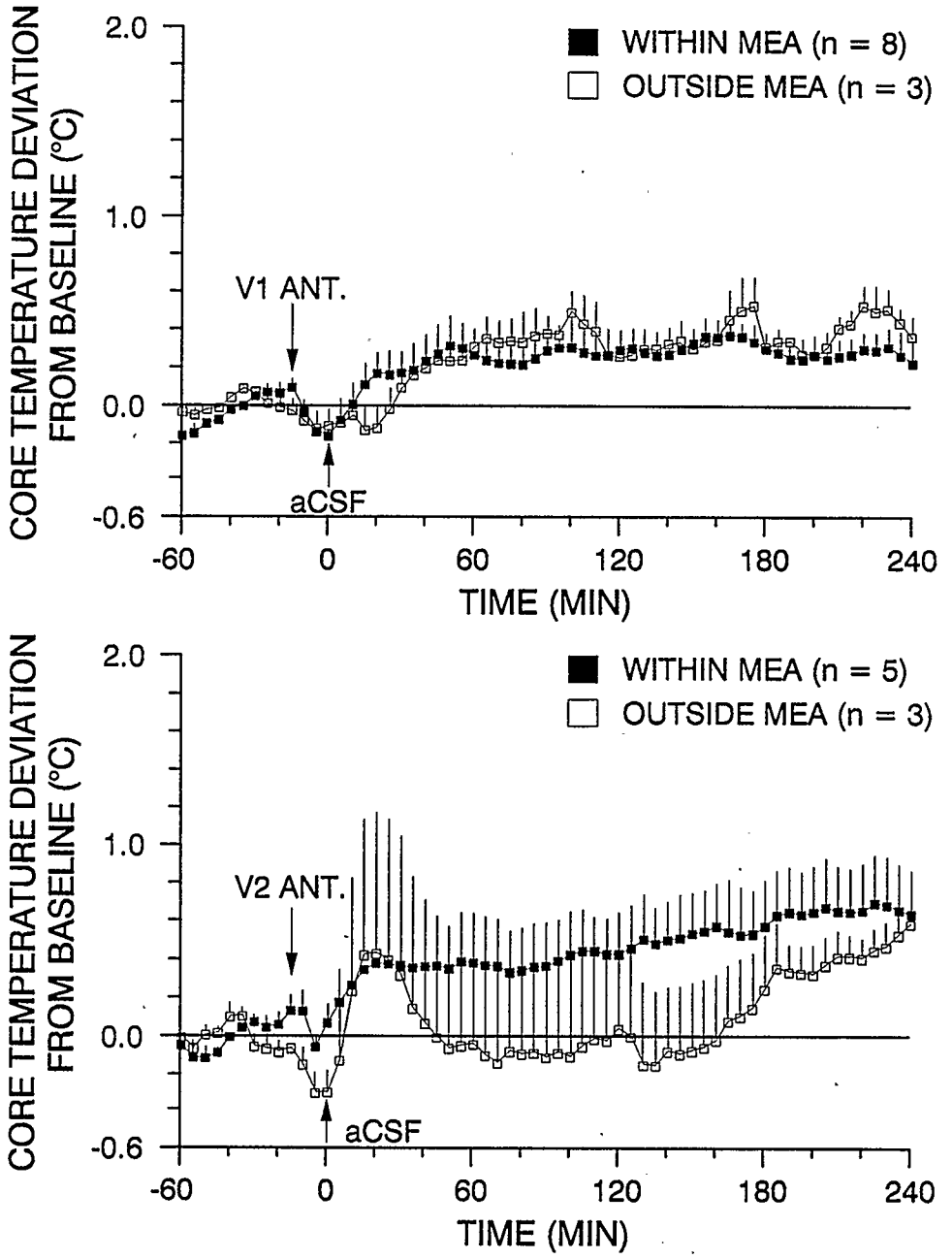


Fig. 20. Top panel: Mean (± S.E.M) temperature responses to the i.c.v. injection of aCSF. Fifteen minutes prior to this, $d(CH_2)_5Tyr(Me)AVP$ (V_{1a} antagonist) was injected into the meA (filled squares) or into sites outside the meA (open squares). Bottom panel: Mean (± S.E.M) temperature responses to the i.c.v. injection of aCSF. Fifteen minutes prior to this, $d(CH_2)_5$-D-ValVAVP (V_2 antagonist) was injected into the meA (filled squares) or into sites outside the meA (open squares) ($P > 0.05$).

Series III

Histological examination of tissue injection sites of the 8 animals used in the third series of experiments showed that tissue injections were localized to the meA and adjacent areas (Fig 21). As previously mentioned, animals were separated on the basis of whether or not tissue injections were localized to the meA on at least one side of the brain. However, since only one animal did not receive an injection within the meA on either side of the brain, data from meA "misses" will not be discussed.

From an average baseline core temperature of 36.8 ± 0.1°C, i.c.v. PGE_1 (50 ng) preceded by aCSF-aCSF (1μl) injection into the meA resulted in an immediate rise in core temperature that reached a maximum of 1.8 ± 0.1°C above baseline within 40 min (n = 7; Fig. 22). However, with aCSF-AVP (40 pmol) pretreatment, i.c.v. PGE_1 evoked hyperthermia that were attenuated to an average peak of 1.1 ± 0.1°C above a baseline of 37.1 ± 0.2°C (P < 0.05; Fig. 22). With $d(CH_2)_5Tyr(Me)AVP$ (400 pmol) - AVP pretreatment, i.c.v. PGE_1 evoked hyperthermia that were significantly greater in magnitude compared to those evoked with aCSF-AVP pretreatment but which were similar to those evoked with aCSF-aCSF pretreatment ($d(CH_2)_5Tyr(Me)AVP$: baseline = 36.9 ± 0.1°C, peak = 1.6 ± 0.2°C; n = 7, P < 0.05; Fig. 22).

Fig. 23 illustrates the effect of DDAVP pretreatment on PGE_1 hyperthermia. Note that the aCSF-aCSF and aCSF-AVP pretreatment controls in Fig. 23 are the same as those used in Fig. 22. From this figure, it was apparent that aCSF-DDAVP pretreatment significantly attenuated the PGE_1-induced hyperthermia. Thus, with aCSF-aCSF pretreatment, the maximum rise in core temperature in response to

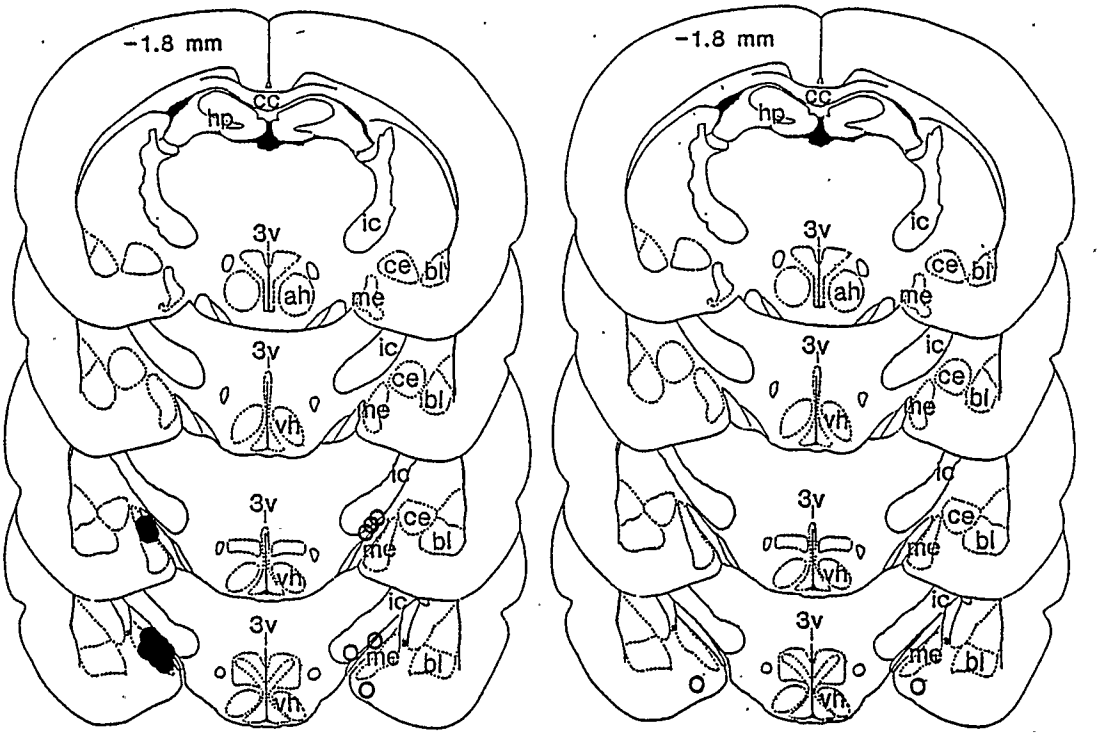


Fig. 21. Schematic histological sections of the rat forebrain showing injection sites that were localized to the meA (filled circles) or to sites surrounding but not impinging upon the meA (open circles) for animals in the third series of experiments. Note that histological data obtained from animals in which tissue injection was localized to the meA on at least one side of the brain are summarized in the left panel. Histological data from animals receiving injections outside the meA on both sides of the brain are summarized in the right panel. For clarity, injection sites localized to the meA are placed on one side of the appropriate histological sections. Abbreviations: 3v, third ventricle; ah, anterior hypothalamus; bl, basolateral amygdaloid nucleus; cc, corpus callosum; ce, central amygdaloid nucleus; hp, hippocampal area; ic, internal capsule; me, medial amygdaloid nucleus; vh, ventromedial hypothalamus.

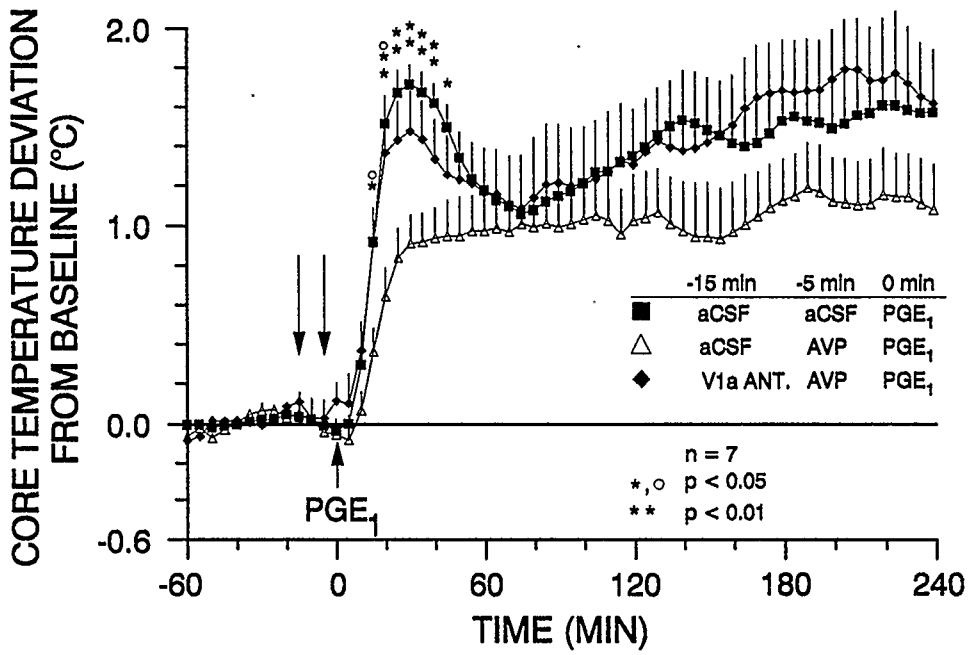


Fig. 22. Mean (± S.E.M) temperature responses to the i.c.v. injection of PGE_1. Five and fifteen minutes prior to this, aCSF or AVP or $d(CH_2)_5Tyr(Me)AVP$ (V_{1a} antagonist) were bilaterally injected into the meA. See figure inset for trial content. Asterisks indicate a significant difference in core temperature deviations between animals receiving aCSF-AVP pretreatment (open triangles) and animals receiving aCSF-aCSF pretreatment (filled squares). Small circles indicate a significant difference in core temperature deviations between animals receiving aCSF-AVP pretreatment (open triangles) and animals receiving V_{1a} antagonist-AVP retreatment (filled diamonds) (*,°$P < 0.05$; **$P < 0.01$).

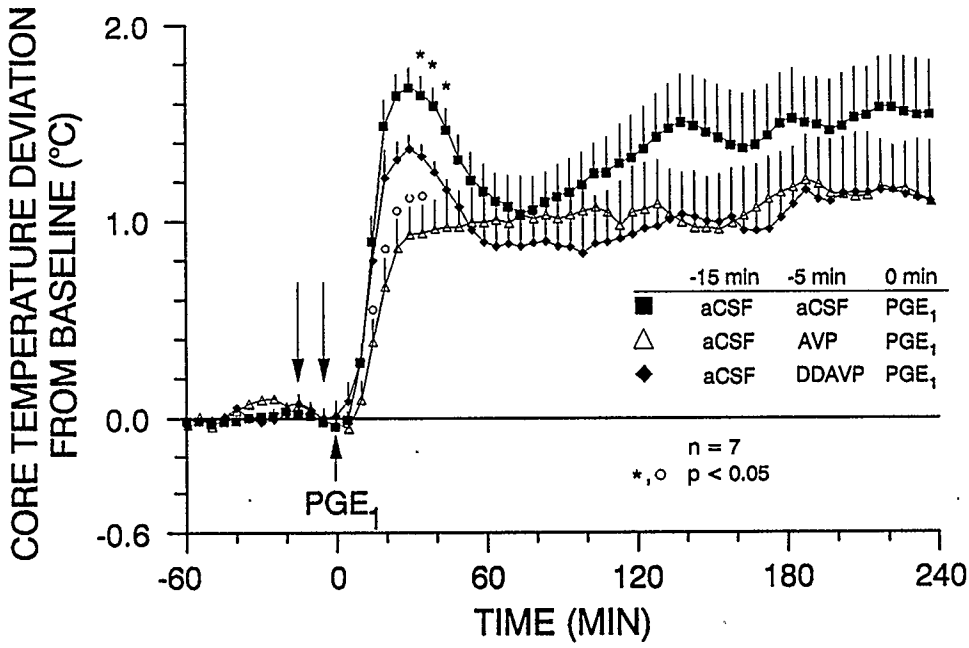


Fig. 23. Mean (± S.E.M) temperature responses to the i.c.v. injection of PGE_1. Five and fifteen minutes prior to this, animals received bilateral injections of aCSF or AVP or DDAVP into the meA. See figure inset for trial content. Asterisks and small circles indicate a significant difference in core temperature deviations between the animals receiving aCSF-DDAVP pretreatment (filled diamonds) and the respective groups (*,°$P < 0.05$).

i.c.v. PGE_1 was 1.8 ± 0.1°C above a baseline of 36.8 ± 0.1°C, while with aCSF-DDAVP pretreatment, the maximal rise in core temperature in response to i.c.v. PGE_1 was attenuated to 1.5 ± 0.1°C above a baseline of 37.2 ± 0.2°C (n = 7; P < 0.05; Fig. 23). In addition, in a pilot study, DDAVP injected into the meA did not significantly alter normothermic core temperature compared to control injection of aCSF (n = 7; data not shown). A comparison of core temperature responses to i.c.v. PGE_1 with aCSF-AVP versus aCSF-DDAVP pretreatment showed that AVP-induced antipyresis was significantly more pronounced than that of DDAVP (P < 0.05; Fig. 23).

D. DISCUSSION

Experiments were undertaken to characterize the central receptor mediating the antipyretic effect of AVP within the meA and to determine if the action of endogenously released AVP is necessary at this site to modulate febrile body temperature.

Intracerebroventricular administration of PGE_1 evoked a short latency rise in core temperature that was maximal within 40 min. Injection of AVP into the meA was found to prevent the normal development of the hyperthermic response to PGE_1. These findings are consistent with earlier work presented in this thesis.

The relatively selective vasopressor (V_{1a}) antagonist, $d(CH_2)_5Tyr(Me)AVP$, was found to prevent the antipyretic action of AVP microinjected into the meA. Since $d(CH_2)_5Tyr(Me)AVP$ alone had no significant effect on afebrile body temperature or on the initial stages of PGE_1 hyperthermia (the time when AVP antipyresis is observed), it is suggested that the $d(CH_2)_5Tyr(Me)AVP$ effects

observed in this study are not due to a non-specific thermogenic action of the antagonist within the meA. This consideration is necessary since $d(CH_2)_5Tyr(Me)AVP$ has been reported to have a non-specific neurodepressant action within the spinal cord (Porter and Brody, 1986). Thus, it is suggested that the central receptors responsible for the antipyretic action of AVP within the meA may resemble those at which AVP elicits pressor effects, namely, V_{1a} receptors. This finding is consistent with anatomical studies demonstrating the presence of V_{1a}-like receptors within the meA (Dorsa et al., 1984; Tribollet et al., 1988; Lawrence et al., 1988) and with studies demonstrating that AVP-induced antipyresis in the VSA is probably mediated by V_{1a}-like receptors (Cooper et al., 1987; Naylor et al., 1988).

The relatively selective antidiuretic (V_2) agonist, DDAVP, was found to be antipyretic since it attenuated PGE_1 hyperthermia while having no significant effect on afebrile core temperature. Thus, it could be suggested that the central receptor mediating AVP-induced antipyresis within the meA may resemble the peripheral V_2 receptor in addition to the V_{1a} receptor. However, since DDAVP-induced antipyresis was significantly weaker than AVP-induced antipyresis, and since DDAVP does have some vasopressor agonistic action (Sawyer et al., 1974), it is therefore possible that the observed DDAVP effects may have been due to interaction with a V_{1a}-like receptor. Also consistent with an argument against V_2 receptor involvement is the finding that V_2 receptor blockade within the meA does not alter the hyperthermic response to i.c.v. PGE_1, whereas V_{1a} receptor blockade augments PGE_1-evoked hyperthermia (see next).

Injection of $d(CH_2)_5Tyr(Me)AVP$ alone into the meA prior to i.c.v. PGE_1 resulted in hyperthermia that were similar to aCSF controls for the first 120 min.

After this, however, instead of returning to baseline levels like the aCSF controls, the core temperature of V_{1a} antagonist pretreated rats became elevated once again and remained high for the duration of the experiment. This relatively long duration of $d(CH_2)_5$Tyr(Me)AVP action is not unusual since other studies report effects resulting from central administration of this antagonist lasting up to 5 hr (Cooper et al., 1987; Naylor et al., 1988; Kruszynski et al., 1980). In addition, the increase in core temperature following V_{1a} antagonist treatment is not likely due to thermogenic effects of this antagonist since it was without significant effect on afebrile core temperature when injected into the meA.

It was also found that PGE_1 hyperthermia were not significantly altered when PGE_1 administration was preceded either by V_{1a} antagonist injection into sites outside the meA, or V_2 antagonist injection into the meA or sites outside the meA. Thus, it appears that the $d(CH_2)_5$Tyr(Me)AVP effects reported in this study are due to a site specific action within the meA on V_{1a}-like receptors. Therefore, an explanation of these data could be that $d(CH_2)_5$Tyr(Me)AVP prevents endogenously released AVP from interacting with its receptors (which may be V_{1a}-like in nature) in the meA, thus preventing AVP from limiting the magnitude and duration of fever. Consistent with this, $d(CH_2)_5$Tyr(Me)AVP was found to block the antipyretic effect of AVP injected into the meA.

Support for the endogenous release of AVP into the meA comes from previous studies demonstrating an increase in AVP immunoreactivity in amygdalar nerve terminals during fever and during the development of pyrogen tolerance in pregnant and nonpregnant animals (Kasting and Martin, 1983; Zeisberger et al., 1983; Zeisberger et al., 1986; Merker et al., 1980; Zeisberger et al., 1981; Cooper

et al., 1988). However, since these studies measured peptide content in nerve terminals and not actual release of the peptide, the timing of AVP release into the meA remains uncertain. Regarding this, it was observed that $d(CH_2)_5Tyr(Me)AVP$ effects on PGE_1 hyperthermia occurred only in the latter stages of the PGE_1 hyperthermia, beginning approximately 120 min after PGE_1 administration. While no conclusions can be reached regarding the timing of endogenous AVP *release* during fever, it is suggested that the *action* of AVP, endogenously released within the meA, may be of greatest importance during the latter (late defervescence) stage in limiting the duration of PGE_1 hyperthermia.

It has been demonstrated earlier that AVP release into the VSA is maximal during fever peak, and as body temperature falls during the defervescence period, AVP release diminishes (Cooper et al., 1979; Ruwe et al., 1985b). Further, it has been shown by the use of V_{1a} antagonists that AVP action in the VSA is necessary during the entire defervescence period to limit the magnitude and duration of fever (Naylor et al., 1988; Cooper et al., 1987). As discussed earlier, AVP action in the meA appears to be of greatest importance only during the latter part of defervescence. Thus, during fever, the timing of AVP action in the meA and VSA appears to differ, but overlap. The significance of this differential timing of AVP action as well as mechanisms regulating AVP release into these sites are unknown.

It can be argued that the results of the third series of experiments are in disagreement with those obtained in the first experimental series which demonstrated that $d(CH_2)_5Tyr(Me)AVP$ pretreatment alone augmented PGE_1 hyperthermia during defervescence. Specifically, in the third experimental series, it was found that $d(CH_2)_5Tyr(Me)AVP$ blocked AVP-induced antipyresis in the meA, but did not

augment PGE_1 hyperthermia during defervescence, therefore contradicting the findings of the first experimental series. However, the trial content of the two experimental series differed in that AVP pretreatment was combined with V_{1a} antagonist pretreatment in the third series but not the first. Thus, a direct comparison between the two experimental series is not justified since drug treatments in the these experimental series were not identical. Nonetheless, it is unclear why V_{1a} antagonist-AVP pretreatment did not also result in augmentation of the late stages of the PGE_1 hyperthermia.

In conclusion, these data support and strengthen the hypothesis that AVP may normally be released within the meA to function as an endogenous antipyretic. In addition, it appears that the antipyretic action of AVP within the meA (like many other central effects of the peptide; Tiberiis et al., 1983; Naylor et al., 1985a; Burnard et al., 1986; Matsuguchi et al., 1982; Naylor et al., 1988; Cooper et al., 1987; Naylor et al., 1987) may be mediated by a V_{1a}-like (vasopressor) receptor. The possibility is not excluded, however, that AVP may exert its effects within the meA by interacting with a receptor that is neither V_{1a} or V_2 in nature.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Experiments were undertaken to investigate the hypothesis that arginine vasopressin (AVP) might function within the medial amygdaloid nucleus (meA) as an endogenous antipyretic.

In the conscious rat, bilateral injection of AVP into the meA evoked drops in core temperature during the plateau phase of interleukin-1 fevers and attenuated hyperthermic responses to the i.c.v. injection of PGE_1. Further, similar injection of AVP in afebrile animals did not alter core temperature. Thus, AVP appears to be an effective antipyretic when injected into the meA of the rat. Furthermore, this antipyretic effect is site specific, being localized to the meA and not surrounding regions.

It was also shown that AVP, when perfused within the meA or VSA, is antipyretic in the urethane-anaesthetized rat. Perfusion of AVP was found to suppress PGE_1-evoked hyperthermia at both sites, though the meA appeared to be less sensitive to AVP antipyretic action than the VSA.

The central receptor mediating AVP-induced antipyresis in the meA was investigated using AVP analogues directed against V_{1a} and V_2 subtypes of vasopressin receptors. Vasopressin suppressed hyperthermia evoked by i.c.v. PGE_1, an effect that was blocked by the V_{1a} antagonist, $d(CH_2)_5Tyr(Me)AVP$. On the other hand, DDAVP, a V_2 receptor agonist, evoked only moderate antipyresis, an effect that was attributed to interaction of this agonist with V_{1a} receptors.

The role of endogenously released AVP in fever suppression was investigated, as it relates to the meA. Bilateral injection of the V_{1a} antagonist,

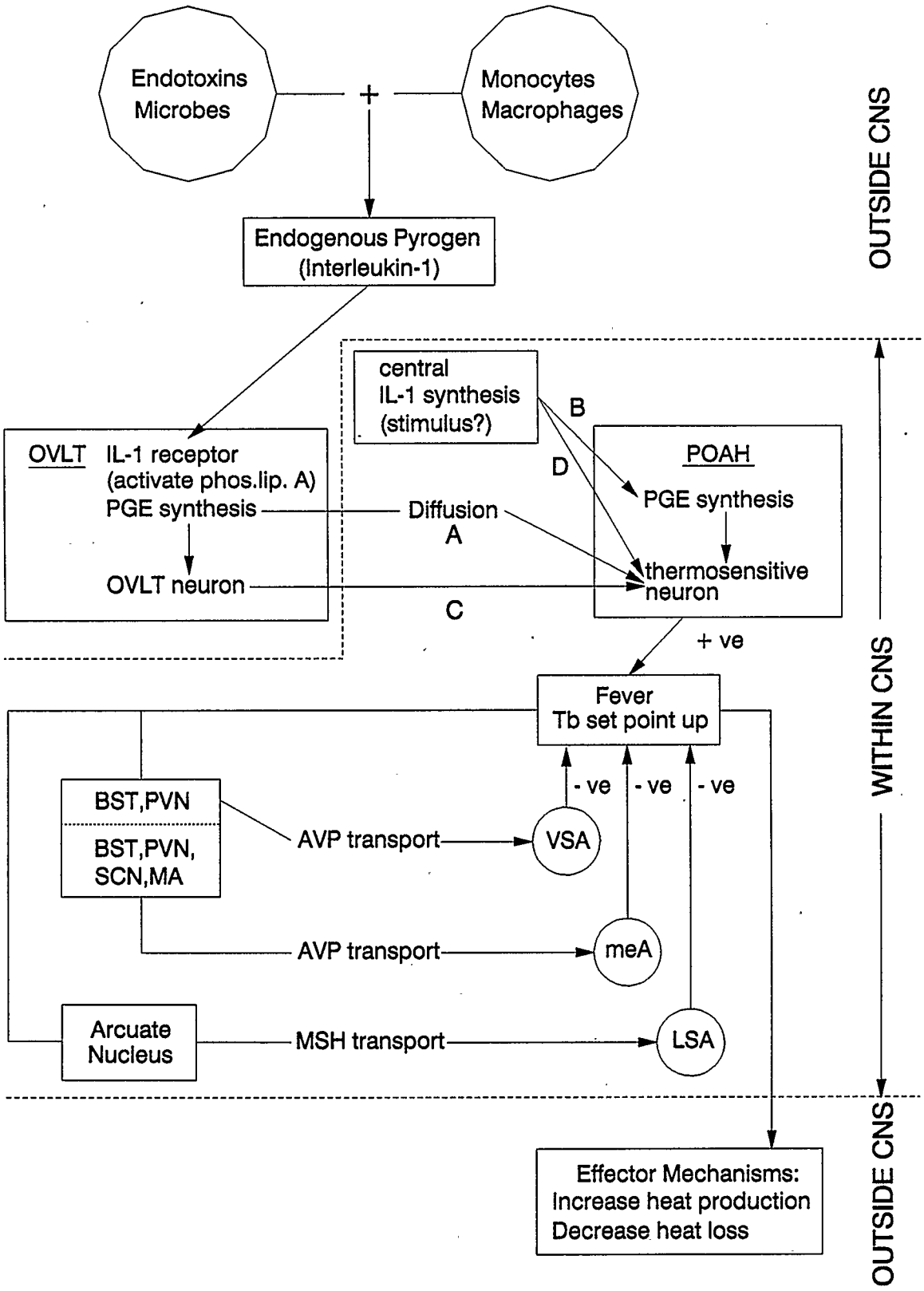
$d(CH_2)_5$Tyr(Me)AVP, into the meA augmented PGE_1 hyperthermia during the latter stages of defervescence. In contrast, similar injection of the V_2 antagonist, $d(CH_2)_5$-D-ValVAVP, did not alter the time course of the PGE_1 hyperthermia. This suggests that endogenously released AVP may act within the meA on a V_{1a}-like receptor to limit the duration of PGE_1 hyperthermia during defervescence.

Thus, these findings support and strengthen the hypothesis that vasopressin might function within the meA as an endogenous antipyretic. Fig. 24 illustrates the component added to the general scheme of fever and antipyresis as a result of research reported in this thesis (compare with Fig. 1).

Earlier studies have shown that AVP microinjected into the VSA evokes, via a sensitization process, convulsive behaviour characterized by myotonic/myoclonic seizures and barrel rotations. Since the amygdala has been implicated in seizure kindling (Wasterlain and Jonec, 1983; Zeisberger et al., 1983) and since AVP is antipyretic in the meA (as in the VSA), it is possible that AVP given into this site, might evoke convulsive behaviour. Indeed, preliminary studies have demonstrated that AVP microinjected into the meA appears to evoke, via a sensitization process, convulsive behaviour (Willcox, personal communication). Thus, as might occur in the VSA, AVP may be involved in the etiology of febrile convulsions through an action in the meA.

The question arises regarding the significance of endogenous antipyresis. It is well known that excessive rises in body temperature can result in permanent CNS damage and, in young children, febrile convulsions. Thus, a negative feedback system that prevents the magnitude of febrile responses from reaching harmfully excessive levels would be beneficial to the host. This suggestion, however, is merely

Fig. 24. Schematic diagram illustrating the sequence of steps in the genesis of fever and components of endogenous antipyretic systems. A new, putative step, the AVP-meA antipyretic system has been added as a result of the research reported in this thesis (compare with Fig. 1). Abbreviations: AVP, arginine vasopressin; AH/POA, anterior hypothalamic/preoptic area; BST, bed nucleus of the stria terminalis; CNS, central nervous system; IL-1, interleukin-1; LSA, lateral septal area; MA, medial amygdala; meA, medial amygdaloid nucleus; MSH, melanocyte stimulating hormone; OVLT, organum vasculosum of the lamina terminalis; PGE, prostaglandin E; PVN, paraventricular nucleus of the hypothalamus; SCN, suprachiasmatic nucleus; Tb, body temperature; VSA, ventral septal area.


Endotoxins
Microbes
+
Monocytes
Macrophages
Endogenous Pyrogen
(Interleukin-1)
OUTSIDE CNS
central
IL-1 synthesis
(stimulus?)
B
D
OVLT
IL-1 receptor
(activate phos.lip. A)
PGE synthesis
Diffusion
A
OVLT neuron
C
POAH
PGE synthesis
thermosensitive
neuron
+ ve
Fever
Tb set point up
WITHIN CNS
- ve
- ve
- ve
BST,PVN
BST,PVN,
SCN,MA
AVP transport
VSA
AVP transport
meA
Arcuate
Nucleus
MSH transport
LSA
Effector Mechanisms:
Increase heat production
Decrease heat loss
OUTSIDE CNS

speculation and has not yet been subjected to experimental test. Related to this, available evidence (on the release and action of AVP) implicates AVP action in the VSA during defervescence to limit the magnitude and duration of fever. Similar evidence from immunocytochemical studies, though not direct, also suggests increased AVP release into the meA during fever. In addition, evidence reported in this thesis implicates AVP action in the meA during the latter stages of defervescence in limiting the duration of fever.

Another aspect of endogenous antipyresis that must be considered is the absence of fever at term in guinea-pigs and sheep. It has been suggested that fever at term may compromise the maternal-newborn bond, thereby resulting in rejection of the newborn by the mother. Additionally, febrile temperatures appear to adversely affect the maturation of lung surfactant (which is important for respiratory function) in the newborn. Thus, it appears that fever at term may be detrimental to newborn animals. Consequently, it appears once again that a negative modulator of fever could be beneficial. Related to this, evidence exists demonstrating the activation of vasopressinergic projections to the amygdala and VSA at term, therefore implicating an endogenous AVP antipyretic system in the development of refractoriness to pyrogens at term. Consistent with this are anatomical studies demonstrating that vasopressinergic neurons in the BST and meA (which project to the VSA and meA) are under hormonal control (De Vries et al., 1985). Clearly, further studies should be undertaken to relate AVP action in the VSA or meA to the absence of fever at term. These and related investigations might provide further insight into the general phenomenon of endogenous antipyresis.

Another physiological event that could involve an endogenous AVP antipyretic system is the development of tolerance to pyrogens following repeated administration of pyrogens. An activation of vasopressinergic projections to the amygdala and VSA has been demonstrated during the development of pyrogen tolerance (Cooper et al., 1988). Further, blocking AVP action in the VSA with a V_{1a} antagonist has been shown to reverse pyrogen tolerance (Wilkinson and Kasting, 1990). Therefore, these findings implicate septal (and perhaps amygdalar) AVP in the development of pyrogen tolerance. Future studies should be directed at examining further the role of septal and amygdalar AVP in the development of pyrogen tolerance.

Currently, it is not known whether the septal AVP antipyretic system functionally interacts with the proposed amygdalar AVP antipyretic system. Indeed, it is possible that these systems function and are regulated independently and are therefore redundant. However, anatomical studies demonstrate extensive interconnections between the two antipyretic systems, thus suggesting that they may be functionally linked in some manner. Future studies should therefore be directed at examining this possibility. Further, it has been shown that the α-MSH endogenous antipyretic system possesses reciprocal connections with the two AVP antipyretic systems. Thus, a possible functional interaction between the different peptide antipyretic systems in endogenous antipyresis must be considered in the future.

It has been reported in this thesis that AVP appears to be less sensitive to AVP antipyretic action than the VSA. This varying efficacy of AVP antipyretic action in the VSA and meA might be due to differences in receptor concentration, distribution area, or AVP binding properties between these sites. Testing this

hypothesis, however, awaits quantitative studies of AVP receptor properties in the meA and VSA. Alternatively, AVP action in the meA might not be critical in the limitation of fevers in nonpregnant adult animals. Consistent with this possibility, is the observation that blocking AVP action (with a V_{1a} antagonist) in the meA during fever in nonpregnant adult rats results in a less profound augmentation of fevers than occurs when AVP action in the VSA is blocked. It is therefore possible that the meA could play a more important role in other endogenous antipyretic processes, namely, the development of pyrogen tolerance in animals at term or in animals subjected to repeated administration of pyrogen. Consistent with this possibility, evidence has been reviewed earlier suggesting that amygdalar AVP may be involved in either of these processes. Consequently, future studies should be directed at examining the possible involvement of amygdalar AVP in either of these processes.

Regardless of whatever the significance of AVP endogenous antipyresis in the meA is, much work is necessary to establish that AVP acts within the meA as a neurotransmitter. The only available data to date suggesting a neurotransmitter role for AVP in the meA is the presence of central AVP synthesis, vasopressinergic nerve terminals, and vasopressin receptors at this site. Clearly, among other things, the following must be demonstrated in order to establish a neurotransmitter role for AVP in the meA; i) AVP release into the meA, ii) inactivation of vasopressin, and iii) actions of vasopressin on meA neurons. Electrophysiological studies are also needed to assess the thermosensitivity and thermoresponsiveness of meA neurons and to examine how intrinsic properties of these neurons change during fever or during the development of pyrogen tolerance.

While many studies focus on the effects and mechanism of action of AVP in endogenous antipyresis, few studies have examined when and how the AVP antipyretic system is activated/deactivated. The use of the urethane-anesthetized rat appears to be a good model to allow investigations of this nature since it is possible with this model to separate pyrogenic from thermal stimuli. This is possible via three physiological manipulations; i) producing fever by administering pyrogen, ii) producing artificial fever by raising body temperature through external heating and iii) administering small doses of pyrogen that are not sufficient to evoke fevers. Initial observations utilizing this approach suggest that a complex interaction of pyrogenic and thermal stimuli may be involved in regulating AVP release into the VSA (Landgraf et al., 1990). However, it still not known whether similar stimuli control AVP release into the meA and whether AVP release into the meA and VSA is coordinately controlled. In addition, mechanisms controlling the timing of central AVP release remain unclear.

The use of "in situ" hybridization in conjunction with the urethane-anesthetized rat could further facilitate the examination of how the AVP endogenous antipyretic system is activated/deactivated. Applied to fever and antipyresis, this technique would allow the direct examination of vasopressin messenger RNA expression in the BST, meA, PVN, and SCN (putative AVP sources of the meA and VSA). Thus, it is possible to specifically determine the effects of pyrogenic and thermogenic stimuli on vasopressin messenger RNA expression in the BST, meA, PVN and SCN. These findings could then be correlated with studies examining the effects of exogenous adminstration of AVP on fever, thermoregulation and neuronal activity.

In closing, the results presented in this thesis support and strengthen the hypothesis that vasopressin might function within the meA in some capacity as an endogenous antipyretic. Whether this endogenous antipyretic system is functionally linked with other similar systems is not known. Further investigation is therefore required to establish a more complete picture of central antipyretic systems which are becoming more complicated and fascinating with each new finding.

LITERATURE CITED

1. Abe, H., Inoue, M., Matsuo, T., and Ogata, N. 1983. The effects of vasopressin on electrical activity in the guinea-pig supraoptic nucleus in vitro. J.Physiol. **337**: 665-685.

2. Alexander, D.P., Bashore, R.A., Britton, R.A., and Forsling, M.A. 1974. Maternal and fetal arginine vasopressin in the chronically catheterized sheep. Biol.Neonate **25**: 242-248.

3. Alexander, S.J., Cooper, K.E., and Veale, W.L. 1987. Blockade of prostaglandin E1 hyperthermia by sodium salicylate given into the ventral septal area of the rat brain. J.Physiol. **384**: 223-231.

4. Anand, B.K., and Brobeck, J.R. 1952. Food intake and spontaneous activity of rats with lesions in the amygdaloid nuclei. J.Neurophysiol. **15**: 421-430.

5. Andersson, B. 1957. Cold defense reactions elicited by electrical stimulation within the septal area of the brain in goats. Acta.Physiol.Scand. **41**: 90-100.

6. Andersson, B., Gale, C.G., Hokfelt, B., and Larsson, B. 1965. Acute and chronic effects of preoptic lesions. Acta.Physiol.Scand. **65**: 45-60.

7. Andersson, B., Grant, R., and Larsson, B. 1956. Central control of heat loss mechanisms in the goat. Acta.Physiol.Scand. **37**: 261-279.

8. Atkins, E., Bodel, P., and Francis, L. 1967. Release of an endogenous pyrogen in vitro from rabbit mononuclear cells. J.Exp.Med. **126**: 357-386.

9. Auron, P.E., Rosenwasser, L.J., Matsushima, K., Copeland, T., Dinarello, C.A., Oppenheim, J.J., and Webb, A.C. 1985. Human and murine interleukin-1 share sequence similarities. J.Mol.Immunol. **2**: 231-239.

10. Axelson, J.F., and van Leeuwen, F.W. 1990. Differential localization of estrogen receptors in vasopressin synthesizing nuclei of the rat brain. J.Neuroendo. **2(2)**: 209-216.

11. Barbour, H.G. 1921. The heat-regulating mechanism of the body. Physiol.Rev. **1**: 295-326.

12. Barney, C.C., Fregly, M.J., Katovich, M.J., and Tyler, P.E. 1979. Effect of cycloheximide on temperature regulation in rats. Brain Res. Bull. **4**: 355-358.

13. Baskin, D.G., Petracca, F.M., and Dorsa, D.M. 1983. Autoradiographic localization of specific binding sites for [3H][Arg8]vasopressin in the septum of the rat brain with tritium- sensitive film. Eur.J.Pharmacol. **90**: 155-157.

14. Beaton, L.E., McKinley, W.A., Berry, C.M., and Ranson, S.W. 1941. Localization of a cerebral centre activating heat-loss mechanisms in monkeys. J.Neurophysiol. **4**: 478-485.

15. Beckstead, R.M. 1979. An autoradiographic examination of corticortical and subcortical projections of the mediodorsal-projection (prefrontal) cortex in the rat. J.Comp.Neurol. **184**: 43-62.

16. Beeson, P.B. 1948. Temperature elevating effect of a substance obtained from polymorphonuclear leukocytes. J.Clin.Invest. **27**: 525-531.

17. Bell, R.C., and Lipton, J.M. 1987. Pulsatile release of antipyretic neuropeptide α-MSH from septum of rabbit during fever. Am.J.Physiol. **252(21)**: R1152-R1157.

18. Bennet, I.L., Petersdorf, R.G., and Keene, W.R. 1957. Pathogenesis of fever: evidence for direct cerebral action of bacterial endotoxin. Trans.Ass.Am.Physns. **70**: 64-72.

19. Bernardini, G.L., Lipton, J.M., and Clark, W.G. 1983. Intracerebroventricular and septal injections of arginine vasopressin are not antipyretic in the rabbit. Peptides **4**: 195-198.

20. Bernheim, H.A., Gilbert, T.M., and Stitt, J.T. 1980. Prostaglandin E levels in third ventricular cerebrospinal fluid of rabbits during fever and changes in body temperature. J.Physiol. **301**: 69-78.

21. Betteridge, A. 1980. Role of Ca^{2++} and cyclic nucleotides in the control of prostaglandin E production in the rat anterior pituitary gland. Biochem.J. **186**: 987-992.

22. Biegon, A., Terlou, M., Voorhuis, T.D., and de Kloet, E.R. 1984. Arginine-vasopressin binding sites in rat brain: a quantitative autoradiographic study. Neurosci.Lett. **44**: 229-234.

23. Blatteis, C.M., Bealer, S.L., Hunter, W.S., Llanos, Q.J., Ahokas, R.A., and Mashburn, T.A., Jr. 1983. Suppression of fever after lesions of the anteroventral third ventricle in guinea pigs. Brain Res. Bull. **11**: 519-526.

24. Blatteis, C.M., Dinarello, C.A., Shibata, H., Llanos-Q, J., Quan, N., and Busija, D.W. 1989. Does circulating interleukin-1 enter the brain?. In: Thermal Physiology. J.B. Mercer, ed. Elsevier, Basel. pp. 385-390.

25. Bodel, P., and Atkins, E. 1967. Release of endogenous pyrogen by human monocytes. New Engl.J.Med. **276**: 1002-1005.

26. Braude, A.I., Carey, F.J., and Zalesky, M. 1955. Studies with radioactive endotoxin. II. Correlation of physiological effects with distribution of radioactivity in rabbits injected with lethal doses of E. Coli endotoxin labelled with radioactive sodium chromate. J.Clin.Invest. **34**: 858-866.

27. Brown, E., Clark, D.L., Roux, V., and Sherman, G.H. 1963. The stimulation of adenosine 3'5'-monophosphate production by antidiuretic factors. J.Biol.Chem. **238**: PC852-P853.(Abstract)

28. Buijs, R.M. 1978. Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat. Pathways to the limbic system, medulla oblongata and spinal cord. Cell Tissue Res. **192**: 423-435.

29. Buijs, R.M., and Swaab, D.F. 1979. Immuno-electron microscopal demonstration of vasopressin and oxytocin synapses in the limbic system of the rat. Cell Tissue Res. **204**: 355-365.

30. Buijs, R.M., Swaab, D.F., Dogterom, J., and van Leeuwen, F.W. 1978. Intra- and extrahypothalamic vasopressin and oxytocin pathways in the rat. Cell Tissue Res. **186**: 423-433.

31. Burnard, D.M., Pittman, Q.J., and Veale, W.L. 1983. Increased motor disturbances in response to arginine vasopressin following hemorrhage or hypertonic saline: evidence for central AVP release in rats. Brain Res. **273**: 59-65.

32. Burnard, D.M., Veale, W.L., and Pittman, Q.J. 1986. Prevention of arginine-vasopressin-induced motor disturbances by a potent vasopressor antagonist. Brain Res. **362**: 40-60.

33. Cabanac, M., Stolwijk, J.A.J., and Hardy, J.D. 1968. Effect of temperature and pyrogens on single unit activity in the rabbit brainstem. J.Appl.Physiol. **24**: 645-652.

34. Caffé, A.R., and van Leeuwen, F.W. 1983. Vasopressin-immunoreactive cells in the dorsomedial hypothalamic region, medial amygdaloid nucleus and locus coeruleus of the rat. Cell Tissue Res. **233**: 23-33.

35. Caffé, A.R., van Leeuwen, F.W., and Luiten, P.G.M. 1987. Vasopressin cells in the medial amygdala of the rat project to the lateral septum and ventral hippocampus. J.Comp.Neurol. **261**: 237-252.

36. Calvert, D.T., and Findlay, J.D. 1975. Localization of the effective thermosensitive site in the preoptic region of the ox. J.Appl.Physiol. **39**: 702-706.

37. Carlisle, H.J. 1969. Effect of preoptic anterior hypothalamic lesion on behavioral thermoregulation in cold. J.Comp.Physiol.Psychol. **69**: 391-402.

38. Chai, C.Y., Lin, M.T., Chen, H.I., and Wang, S.C. 1971. The site of action of leukocytic pyrogen and antipyresis of sodium acetylsalicylate in monkeys. Neuropharm. **10**: 715-723.

39. Chossat, C. 1820. Extrait d'un memoire sur l'influence du systeme nerveux sur la chaleur animale. Ann. Chim. Phys. **15**: 37-39. (From Lomax, 1979).

40. Clark, G., Magoun, H.W., and Ranson, S.W. 1939. Hypothalamic regulation of body temperature. J.Neurophysiol. **2**: 61-80.

41. Clark, W.G., and Cumby, H.R. 1975. The antipyretic effect of indomethacin. J.Physiol. **248**: 625-638.

42. Coceani, F., Bishai, I., Dinarello, C.A., and Fitzpatrick, F.A. 1983. Prostaglandin E2 and thromboxane B2 in cerebrospinal fluid of afebrile and febrile cat. Am.J.Physiol. **244**: R785-R793.

43. Conrad, L.C.A., and Pfaff, D.W. 1976a. Efferents from medial basal forebrain and hypothalamus in the rat. I. An autoradiographic study of the medial preoptic area. J.Comp.Neurol. **169**: 185-220.

44. Conrad, L.C.A., and Pfaff, D.W. 1976b. Efferents from medial basal forebrain and hypothalamus in the rat. II. An autoradiographic study of the anterior hypothalamus. J.Comp.Neurol. **169**: 221-262.

45. Cooper, K.E. 1987. The neurobiology of fever: thoughts on recent developments. Annu.Rev.Neurosci. **10**: 297-324.

46. Cooper, K.E., and Cranston, W.I. 1963. Clearance of radioactive bacterial pyrogen from the circulation. J.Physiol. **166**: 41-42P.(Abstract)

47. Cooper, K.E., and Veale, W.L. 1974. Fever, an abnormal drive to the heat conserving and producing mechanisms?. In: Recent Studies of Hypothalamic Function. K. Lederis and K.E. Cooper, eds. Karger, Basel. pp. 391-398.

48. Cooper, K.E., Blähser, S., Malkinson, T.J., Merker, G., Roth, J., and Zeisberger, E. 1988. Changes in body temperature and vasopressin content of brain neurons, in pregnant and non-pregnant guinea pigs, during fevers produced by Poly I:Poly C. Pflügers.Arch. **412**: 292-296.

49. Cooper, K.E., Cranston, W.I., and Honour, A.J. 1967. Observations on the site and mode of action of pyrogens in the rabbit brain. J.Physiol. **191**: 325-337.(Abstract)

50. Cooper, K.E., Cranston, W.I., and Snell, E.S. 1964. Temperature regulation during fever in man. Clin.Sci. **27**: 345-365.

51. Cooper, K.E., Kasting, N.W., Lederis, K., and Veale, W.L. 1979. Evidence supporting a role for vasopressin in natural suppression of fever in sheep. J.Physiol. **295**: 33-45.

52. Cooper, K.E., Naylor, A.M., and Veale, W.L. 1987. Evidence supporting a role for endogenous vasopressin in fever suppression in the rat. J.Physiol. **387**: 163-172.

53. Cooper, K.E., Veale, W.L., and Pittman, Q.J. 1976. The pathogenesis of fever. In: Brain Disfunction in Infantile Febrile Convulsions. M.A.B. Brazier and F. Coceani, eds. Raven, New York. pp. 107-115.

54. Cranston, W.I., Dawson, N.J., Hellon, R.F., and Townsend, Y. 1978. Contrasting actions of cycloheximide on fever caused by arachidonic acid and by pyrogen. J.Physiol. **285**: 35P-35P.(Abstract)

55. Cranston, W.I., Duff, G.W., Hellon, R.F., Mitchell, D., and Townsend, Y. 1976. Evidence that brain prostaglandin synthesis is not essential for fever. J.Physiol. **259**: 239-249.

56. Cranston, W.I., Hellon, R.F., Luff, R.H., Rawlins, M.D., and Rosendorff, C. 1970. Observations on the mechanism of salicylate-induced antipyresis. J.Physiol. **210**: 593-600.

57. Cranston, W.I., Hellon, R.F., and Mitchell, D. 1975. A dissociation between fever and prostaglandin concentration in cerebrospinal fluid. J.Physiol. **253**: 583-592.

58. Cranston, W.I., Hellon, R.F., and Mitchell, D. 1980. Suppression of fever in rabbits by a protein synthesis inhibitor, anisomycin. J.Physiol. **305**: 337-344.

59. Cranston, W.I., Hellon, R.F., and Townsend, Y. 1982. Further observations on the suppression of fever in rabbits by intracerebral action of anisomycin. J.Physiol. **322**: 441-445.

60. Crawshaw, L.I., and Stitt, J.T. 1975. Behavioral and autonomic induction of prostaglandin E_1 fever in squirrel monkeys. J.Physiol. **244**: 197-206.

61. Currie, J. 1798. Medical Reports on the Effects of Water, Cold and Warm as a Remedy in Fever and Other Diseases, Vol. 1., Caldwell and Davies, London. (From Lomax, 1979).

62. Dascombe, M.J. 1985. The pharmacology of fever. Prog.Neurobiol. **25**: 327-373.

63. Dascombe, M.J., and Milton, A.S. 1979. Study on the possible entry of bacterial endotoxin and prostaglandin E_2 into the central nervous system from the blood. Br.J.Pharm. **66**: 565-572.

64. de Olmos, J.S., and Carrer, H. 1978. A horseradish peroxidase study of the afferent connections of the medial basal hypothalamus in the rat. Anat.Rec. **190**: 380-380.

65. de Olmos, J.S., Alheid, G.F., and Beltramino, C.A. 1985. Amygdala. In: The Rat Nervous System (vol. 1): Forebrain and Midbrain. G. Paxinos, ed. Academic Press, Toronto. pp. 223-334.

66. De Vries, G.J., and Buijs, R.M. 1983. The origin of the vasopressinergic and oxytocinergic innervation of the rat brain with special reference to the lateral septum. Brain Res. **273**: 307-317.

67. De Vries, G.J., Buijs, R.M., van Leeuwen, F.W., Caffé, A.R., and Swaab, D.F. 1985. The vasopressinergic innervation of the brain in normal and castrated rats. J.Comp.Neurol. **233**: 236-254.

68. De Wied, D., Gaffori, O., Van Ree, J.M., and De Jong, W. 1984. Vasopressin antagonists block peripheral as well as central vasopresin receptors. Pharmacol. Biochem. Behav. **21**: 393-400.

69. Dinarello, C.A. 1984. Interleukin-1. Rev.Infect.Dis. **6**: 51-95.

70. Dinarello, C.A., and Bernheim, H.A. 1981. Ability of human leukocytic pyrogen to stimulate brain prostaglandin synthesis in vitro. J.Neurochem. **37**: 702-708.

71. Dinarello, C.A., Bodel, P., and Atkins, E. 1968. The role of the liver in the production fever and in pyrogen tolerance. Trans.Assoc.Am.Phys. **81**: 334-344.

72. Dinarello, C.A., Weiner, P., and Wolff, S.M. 1978. Radiolabelling and disposition in rabbits of purified human leukocyte pyrogen. Clin.Res. **26**: 522A-522A.(Abstract)

73. Disturnal, J.E., Veale, W.L., and Pittman, Q.J. 1985. Electrophysiological analysis of potential arginine vasopressin projections to the ventral septal area of the rat. Brain Res. **342**: 162-167.

74. Disturnal, J.E., Veale, W.L., and Pittman, Q.J. 1986. The ventral septal area: electrophysiological evidence for putative arginine vasopressin projections onto thermoresponsive neurons. Neuroscience. **19**: 795-802.

75. Disturnal, J.E., Veale, W.L., and Pittman, Q.J. 1987. Modulation by arginine vasopressin of glutamate excitation in the ventral septal area of the rat brain. Can.J.Physiol.Pharmacol. **65**: 30-35.

76. Divac, I. 1979. Patterns of subcortical-cortical projections as revealed by somatopetal horseradish-peroxidase tracing. Neurosci. **4**: 455-461.

77. Dorsa, D.M., Majumdar, L.A., Petracca, F.M., Baskin, D.G., and Cornett, L.E. 1983. Characterization and localization of 3H-arginine8-vasopressin binding to rat kidney and brain tissue. Peptides **4**: 699-706.

78. Dorsa, D.M., Petracca, F.M., Baskin, D.G., and Cornett, L.E. 1984. Localization and characterization of vasopressin-binding sites in the amygdala of the rat brain. J.Neurosci. **4(7)**: 1764-1770.

79. Dubois, E.F. 1949. Why are fever temperatures over 106°F rare?. Am.J.Med.Sci. **217**: 361-368.

80. Eisenman, J.S. 1969. Pyrogen induced changes in thermosensitivity of septal and preoptic neurons. Am.J.Physiol. **216**: 330-334.

81. Eisenman, J.S. 1974. Depression of thermosensitivity by bacterial pyrogen in rabbits. Am.J.Physiol. **227**: 1067-1073.

82. Eisenman, J.S. 1982. Electrophysiology of the anterior hypothalamus: thermoregulation and fever. In: Pyretic and Antipyretics. Handbook of Experimental Pharmacology, Vol. 60. A.S. Milton, ed. Springer-Verlag, Berlin. pp. 187-217.

83. Eskay, R.L., Giraud, P., Oliver, C., and Brownstein, M.J. 1979. Distribution of α-melanocyte-stimulating hormone in the rat brain: evidence that α-MSH-containing cells in the arcuate region send projections to extrahypothalamic areas. Brain Res. **178**: 55-67.

84. Federico, P., and Veale, W.L. 1990. Infusion of arginine vasopressin into the medial nucleus of the amygdala attenuates interleukin-1 fevers in the rat. Can.J.Physiol.Pharmacol. **68(5)**: Aix-Ax.(Abstract)

85. Feldberg, W., and Gupta, K.P. 1973. Pyrogen fever and prostaglandin activity in cerebrospinal fluid. J.Physiol. **228**: 41-53.

86 Feldberg, W., and Saxena, P.N. 1971. Further studies on prostaglandin E_1 fever in cats. J.Physiol. **219**: 739-745.(Abstract)

87 Feldberg, W., Gupta, K.P., Milton, A.S., and Wendlandt, S. 1973. Effect of pyrogen and antipyretics on prostaglandin activity in cisternal c.s.f. of unanaesthetized cats. J.Physiol. **234**: 279-303.

88. Flower, R.J, and Vane, J.R. 1972. Inhibition of prostaglandin synthetase in brain explains the antipyretic activity of paracetamol (4-acetamidophenol). Nature **240**: 410-411.

89. Fontana, A., Hengartner, H., de Tribolet, N., and Weber, E. 1984a. Glioblastoma cells release interleukin 1 and factors inhibiting interleukin 2-mediated effects. J.Immunol. **132**: 1837-1844.

90. Fontana, A., Kristenson, F., Dubs, R., Gemsa, D., and Weber, E. 1982. Production of prostaglandin E and interleukin-1 like factor by cultured astrocytes and C_6 glioma cells. J.Immunol. **129**: 2413-2419.(Abstract)

91. Fontana, A., Weber, E., and Dayer, J.M. 1984b. Synthesis of interleukin 1/endogenous pyrogen in the brain of endotoxin-treated mice: a step in fever induction?. J.Immunol. **133**: 1696-1698.

92. Ford, D.M. 1974. A selective action of prostaglandin E_1 on hypothalamic neurons in the cat which respond to brain cooling. J.Physiol. **242**: 142P-143P.(Abstract)

93. Freund-Mercier, M.J., Dietl, M.M., and Stoeckel, M.E. 1988a. Quantitative autoradiographic mapping of neurohypophyseal hormone binding sites in the rat forebrain and pituitary gland-II. Comparative study on the Long-Evans and Brattleboro strains. Neurosci. **26(1)**: 273-281.

94. Freund-Mercier, M.J., Stoeckel, M.E., Dietl, M.M., Palacios, J.M., and Richard, P. 1988b. Quantitative autoradiographic mapping of neurohypophyseal hormone binding sites in the rat forebrain and pituitary gland-I. Characterization of different binding sites and their distribution in the Long-Evans strain. Neurosci. **26(1)**: 261-272.

95. Fyda, D.M., Cooper, K.E., and Veale, W.L. 1989. Indomethacin-induced antipyresis in the rat: role of vasopressin receptors. Brain Res. **494**: 307-314.

96. Fyda, D.M., Mathieson, W.B., Cooper, K.E., and Veale, W.L. 1990. The effectiveness of arginine vasopressin and sodium salicylate as antipyretics in the Brattleboro rat. Brain Res. **512**: 243-247.

97. Galanos, C., Rietschel, E.T., Luderitz, O., and Westphal, O. 1972. Biological activities of lipid A complexed with bovine serum albumin. Eur.J.Biochem. **31**: 230-233.

98. Garris, D.R. 1979. Direct septo-hypothalamic projections in the rat. Neurosci.Lett. **13**: 83-90.

99. Glyn, J.R., and Lipton, J.M. 1981. Hypothermic and antipyretic effects of centrally administered ACTH (1-24) and α-melanotropin. Peptides **2**: 177-187.

100. Glyn-Ballinger, J.R., Bernardini, G.L., and Lipton, J.M. 1983. α-MSH injected into the septal region reduces fever in rabbits. Peptides **4**: 199-203.

101. Gordon, C.J., and Heath, J.E. 1979. The effect of prostaglandin E_1 on the firing rate of thermally sensitive and insensitive neurons in the preoptic/anterior hypothalamus of unanaesthetized rabbits. Fed.Proc. **38**: 1295.(Abstract)

102. Haberly, L.B., and Price, J.L. 1978. Association and commissural fiber systems of the olfactory cortex of the rat. I. Systems originating in the piriform cortex and adjacent areas. J.Comp.Neurol. **178**: 711-740.

103. Hales, J.R.S., Bennet, J.W., and Fawcett, A.A. 1973. Thermoregulatory effects of prostaglandin E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$ in the sheep. Pflügers.Arch. **339**: 125-133.

104. Hemingway, A., Forgrave, P., and Birzis, L. 1954. Shivering suppression by hypothalamic stimulation. J.Neurophysiol. **17**: 375-386.

105. Holdeman, M., Khorram, O., Samson, W.K., and Lipton, J.M. 1985. Fever-specific changes in central MSH and CRF concentrations. Am.J.Physiol. **248**: R125-R129.

106. Hoo, S.L., Lin, M.T., Wei, R.D., Chai, C.Y., and Wang, S.C. 1972. Effects of sodium acetylsalicylate on the release of pyrogen from leukocytes. Proc.Soc.Exp.Biol.Med. **139**: 1155-1158.

107. Hoorneman, E.M.D., and Buijs, R.M. 1982. Vasopressin fiber pathways in the rat brain following suprachiasmatic nucleus lesioning. Brain Res. **243**: 235-241.

108. Hori, T., Nakashima, T., Kiyohara, T., and Shibata, M. 1984. Effects of leukocytic pyrogen and sodium salicylate on hypothalamic thermosensitive neurons in vitro. Neurosci.Lett. **49(3)**: 313-318.

109. Isenschmid, R., and Schnitzler, W. 1914. Beitrag zur lokalisation des der warmeregulation vorstehenden zentralapparates im zwischenhirn. Arch. Exp. Pathol. Pharmakol. **76**: 202-223. (From Lomax, 1979).

110. IUPS Thermal Commission 1987. Glossary of terms for thermal physiology. Pflügers.Arch. **410**: 567-587.

111. Jackson, D.L. 1967. A hypothalamic region responsive to localized injection of pyrogens. J.Neurophysiol. **30**: 586-602.

112. Jard, S., Gaillard, R.C., Guillon, G., Marie, J., Schoenenberg, P., Muller, A.F., Manning, M., and Sawyer, W.H. 1986. Vasopressin antagonists allow demonstration of a novel type of vasopressin receptor in the rat adenohypophysis. Mol.Pharmacol. **30**: 171-177.

113. Jell, R.M., and Sweatman, P. 1977. Prostaglandin-sensitive neurones in cat hypothalamus: relation to thermoregulation and to biogenic amines. Can.J.Physiol.Pharmacol. **55**: 560-567.

114. Joels, M., and Urban, I.J.A. 1982. The effect of microiontophoretically applied vasopressin and oxytocin on single neurones in the septum and dorsal hippocampus of the rat. Neurosci.Lett. **33**: 79-84.

115. Kamdasamy, S.B., and Williams, B.A. 1984. Hypothermic and antipyretic effects of ACTH (1-24) and α-melanotropin in guinea-pigs. Neuropharm. **23(1)**: 49-53.

116. Kasting, N.W. 1980. An Antipyretic System in the Brain and the Role of Vasopressin. Ph.D. Thesis, University of Calgary.

117. Kasting, N.W. 1986. Potent stimuli for vasopressin release, hypertonic saline and hemorrhage, cause antipyresis in the rat. Regul.Pept. **15**: 293-300.

118. Kasting, N.W., and Martin, J.B. 1983. Changes in immunoreactive vasopressin concentrations in brain regions of the rat in response to endotoxin. Brain Res. **258**: 127-132.

119. Kasting, N.W., and Wilkinson, M.F. 1986. An antagonist to the antipyretic effects of intracerebroventricularly administered vasopressin in the rat. In: Homeostasis and Thermal Stress. K.E. Cooper, P. Lomax, E. Schönbaum and W.L. Veale, eds. Karger, Basel. pp. 137-139.

120. Kasting, N.W., Veale, W.L., and Cooper, K.E. 1978. Suppression of fever at term of pregnancy. Nature **271**: 245-246.

121. Kasting, N.W., Veale, W.L., and Cooper, K.E. 1979a. Antipyresis following perfusion of brain sites with vasopressin. Experientia **35**: 208-209.

122. Kasting, N.W., Veale, W.L., and Cooper, K.E. 1979b. Endogenous pyrogen release by fetal sheep and pregnant sheep leukocytes. Can.J.Physiol.Pharmacol. **57**: 1453-1456.

123. Kasting, N.W., Veale, W.L., and Cooper, K.E. 1980. Convulsive and hypothermic effects of vasopressin in the brain of the rat. Can.J.Physiol.Pharmacol. **58**: 316-319.

124. Kasting, N.W., Veale, W.L., Cooper, K.E., and Lederis, K. 1981. Vasopressin may mediate febrile convulsions. Brain Res. **213**: 327-333.

125. Kawakami, M., Seto, M., Negoro, H., Yamaoka, S., and Hohri, M. 1972. Participation of limbic-hypothalamic structures in cold adaptation. In: Advances in Climatic Physiology. S. Itoh, K. Ogata and H. Yoshimura, eds. Karger, Basel. pp. 219-246.

126. Kelly, A.R., Domesick, V.H., and Nauta, W.J.H. 1982. The amygdalostriatal projection in the rat, an anatomical study by anterograde and retrograde tracing methods. Neurosci. **7**: 615-630.

127. Kirk, C.J., Rodriques, L.M., and Hems, D.A. 1979. The influence of vasopressin and related peptides on glycogen phosphorylase activity and phosphatidylinositol metabolism in hepatocytes. Biochem.J. **178**: 493-496.

128. Klempner, M.S., Dinarello, C.A., and Gallin, J.I. 1978. Human leukocytic pyrogen induces release of specific granule contents from human neutrophils. J.Clin.Invest. **61**: 1330-1336.

129. Kluger, M.J. 1980. Historical aspects of fever and its role in disease. In: Thermoregulatory Mechanisms and their Therapeutic Implications. B. Cox, P. Lomax, A.S. Milton and E. Schönbaum, eds. Karger, Basel. pp. 65-70.

130. Koigegami, H., Kushiro, H., and Kimoto, A. 1952. Army Med. Res. Lab. Rep. **6**: 76. (From Kawakami et al., 1972).

131. Kovacs, G.L., and De Wied, D. 1983. Hormonally active vasopressin suppresses endotoxin-induced fever in rats. Lack of effect of oxytocin and a behaviorally active vasopressin fragment. Neuroend. **37**: 258-261.

132. Krettek, J.E., and Price, J.L. 1977. The cortical projections of the medial dorsal nuclei and adjacent thalamic nuclei in the rat. J.Comp.Neurol. **171**: 157-193.

133. Krettek, J.E., and Price, J.L. 1978. Amygdaloid projections to subcortical structures within the basal forebrain and brainstem in the rat and cat. J.Comp.Neurol. **178**: 225-254.

134. Krieger, M.S., Conrad, L.C.A., and Pfaff, D.W. 1979. An autoradiographic study of the efferent connections of the ventromedial nucleus of the hypothalamus. J.Comp.Neurol. **183**: 785-816.

135. Kruk, Z.L., and Brittain, R.T. 1972. Changes in body, core and skin temperature following intracerebroventricular injection of substances in the conscious rat: interpretation of data. J.Pharm.Pharmac. **24**: 835-837.

136. Kruse, H., Van Wimersma Greidanus, Tj.B., and De Wied, D. 1977. Barrel rotation induced by vasopressin and related peptides in rats. Pharmacol.Biochem.Behav. **7**: 311-313.

137. Kruszynski, M., Lammek, B., and Manning, M. 1980. [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),2-(O-methyl)tyrosine]arginine-vasopressin and [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)]arginine-vasopressin, two highly potent antagonists of the vasopressor response to arginine vasopressin. J.Med.Chem. **23**: 364-368.

138. Laburn, H.P., Mitchell, D., and Rosendorff, C. 1977. Effect of prostaglandin antagonism on sodium arachidonate fever in rabbits. J.Physiol. **267**: 559-570.

139. Landgraf, R., Malkinson, T.J., Veale, W.L., Lederis, K., and Pittman, Q.J. 1990. Vasopressin and oxytocin in the rat brain in response to prostaglandin fever. Am.J.Physiol. (In Press)

140. Lawrence, J.A., Poulin, P., Lawrence, D., and Lederis, K. 1988. [3H]arginine vasopressin binding to rat brain: a homogenate and autoradiographic study. Brain Res. **446**: 212-218.

141. Leibermaster, C. 1871. Ueber Warme-Regulierung und Fieber. Heft 19, Sammlung Klinischer Vortage in Verbindung mit deutschen Klinikern, herausgegeben von Richard Volkmann, Breitkopf und Hartel, Leipzig. (From Naylor, 1987).

142. LeMay, L.G., Vander, A.J., and Kluger, M.J. 1990. Role of interleukin 6 in fever in rats. Am.J.Physiol. **258**: R798-R803.

143. Lin, M.T., and Chai, C.Y. 1972. The antipyretic effect of sodium salicylate on pyrogen induced fever in the rabbit. J.Pharm.Exp.Ther. **180**: 603-609.

144. Lipton, J.M., and Kennedy, J.I. 1979. Central thermosensitivity during fever produced by intra POAH and intravenous injection of pyrogen. Brain Res.Bull. **4**: 23-34.

145. Lomax, P. 1979. Historical development of concepts of thermoregulation. In: Body temperature. P. Lomax and E. Schönbaum, eds. Dekker, New York. pp. 1-23.

146. Long, N.C., Vander, A.J., Kunkel, S.L., and Kluger, M.J. 1990. Antiserum against tumor necrosis factor increases stress hyperthermia in rats. Am.J.Physiol. **258**: R591-R595.

147. Luckasen, J.R., White, J.G., and Kersey, J.H. 1974. Mitogenic properties of a calcium ionophore, A23187. Proc.Natl.Acad.Sci.U.S.A. **71**: 5088-5090.

148. Luderitz, O., Westphal, O., Staub, A.M., and Nikaido, H. 1971. Isolation and chemical immunological characterization of bacterial lipopolysaccharides. In: Microbial Toxins. Vol. 4. G. Weinbaum, S. Kadis and S.J. Ajl, eds. Academic Press, New York. pp. 145-233.

149. Luiten, P.G.M., Ono, T., Nishijo, H., and Fukuda, M. 1983. Differential input from the amygdaloid body to the ventromedial hypothalamic nucleus of the rat. Neurosci.Lett. **35**: 253-258.

150. Luskin, M.B., and Price, J.L. 1983. The topographic organization of associational fibers of the olfactory system in the rat, including centrifugal fibers to the olfactory bulb. J.Comp.Neurol. **216**: 264-291.

151. MacPherson, R.K. 1959. The effect of fever on temperature regulation in man. Clin.Sci. **18**: 281-287.

152. Malkinson, T.J., Bridges, T.E., Lederis, K., and Veale, W.L. 1987. Perfusion of the septum of the rabbit with vasopressin antiserum enhances endotoxin fever. Peptides **8**: 385-389.

153. Malkinson, T.J., Cooper, K.E., and Veale, W.L. 1988. Physiological changes during thermoregulation and fever in urethan-anesthetized rats. Am.J.Physiol. **255**: R73-R81.

154. Malkinson, T.J., Cooper, K.E., and Veale, W.L. 1990. Cerebrospinal fluid pressure in conscious rats during prostaglandin E1 fever. Am.J.Physiol. **258**: R783-R787.

155. Manning, M., Nawrocka, E., Misicka, A., Olma, A., Klis, W.A., Seto, J., and Sawyer, W.H. 1984. Potent and selective antagonists of the antidiuretic responses to arginine-vasopressin based on modifications of [1-(beta-mercapto-beta,beta-pentamethylenepropionic acid),2-D-isoleucine,4-valine]arginine-vasopressin at position 4. J.Med.Chem. **27**: 423-49.

156. Martin, S.M., Malkinson, T.J., Veale, W.L., and Pittman, Q.J. 1990. Depletion of brain alpha-MSH alters prostaglandin and interleukin fever in rats. Brain Res. (In Press)

157. Mathieson, W.B., Federico, P., Veale, W.L., and Pittman, Q.J. 1989b. Single-unit activity in the bed nucleus of the stria terminalis during fever. Brain Res. **486**: 49-55.

158. Mathieson, W.B., Pittman, Q.J., and Veale, W.L. 1989a. Ventral septal neurons may mediate vasopressin antipyresis by altering activity of arcuate and dorsomedial hypothalamic neurons. Soc.Neurosci. **15**: 91.10-91.10.(Abstract)

159. Matsuguchi, H., Sharabi, F.M., Gordon, F.J., Johnson, A.K., and Schmid, P.G. 1982. Blood pressure and heart rate responses to microinjection of vasopressin into the nucleus tractus solitarus region of the rat. Neuropharm. **21**: 687-693.

160. Meisenberg, G., and Simmons, W.H. 1984. Hypothermia induced by centrally administered vasopressin in rats. A structure-activity study. Neuropharm. **23**: 1195-1200.

161. Merker, G., Blähser, S., and Zeisberger, E. 1980. Reactivity pattern of vasopressin-containing neurons and its relation to the antipyretic reaction in the pregnant guinea pig. Cell Tissue Res. **212**: 47-61.

162. Merker, G., Roth, J., Zeisberger, E. 1989. Thermoadaptive influence on reactivity pattern of vasopressinergic neurons in the guinea pig. Experientia **45**: 722-726.

163. Milton, A.S. 1982. Prostaglandins in fever and the mode of action of antipyretic drugs. In: Pyretics and Antipyretics. Handbook of Experimental Pharmacology, Vol. 60. A.S. Milton, ed. Springer-Verlag, Berlin. pp. 257-303.

164. Milton, A.S., and Sawheny, V.K. 1980. Protein synthesis and fever. In: Pyretics and Antipyretics. Handbook of Experimental Pharmacology. Vol. 60. A.S. Milton, ed. Springer Verlag, Berlin. pp. 305-315.

165. Milton, A.S., and Wendlandt, S. 1971. Effects on body temperature of prostaglandins of the A, E and F series on injection into the third ventricle of unanaesthetized cats and rabbits. J.Physiol. **218**: 325-336.

166. Mitchell, R.H., Kirk, C.J., and Billah, M.M. 1979. Hormonal stimulation of phosphatidylinositol breakdown, with particular reference to the hepatic effects of vasopressin. Biochem.Soc.Trans. **7**: 861-865.

167. Murabe, Y., Nishia, K., and Sano, Y. 1981. Cells capable of uptake of horseradish peroxidase in some circumventricular organs of the cat and the rat. Cell Tissue Res. **219**: 85-92.

168. Murphy, M.T., Richards, D.B., and Lipton, J.M. 1983. Antipyretic potency of centrally administered α-melanocyte stimulating hormone. Science **221**: 192-192.

169. Myers, R.D. 1971. Methods for chemical stimulation of the brain. In: Methods in Psychobiology. R.D. Myers, ed. Academic Press, New York. pp. 247-280.

170. Myers, R.D., and Tyrell, M. 1972. Fever: Reciprocal shift in brain sodium to calcium ratio as the set-point temperature rises. Science **178**: 765-767.

171. Myers, R.D., and Veale, W.L. 1971. The role of sodium and calcium ions in the hypothalamus in the control of body temperature of the unanaesthetized cat. J.Physiol. **212**: 411-430.

172. Naylor, A.M. 1987. Central Vasopressin and Endogenous Antipyresis. Ph.D. Thesis, University of Calgary.

173. Naylor, A.M., Gubitz, G.J., Dinarello, C.A., and Veale, W.L. 1987. Central effects of vasopressin and 1-desamino-8-D-arginine vasopressin (DDAVP) on interleukin-1 fever in the rat. Brain Res. **401**: 173-177.

174. Naylor, A.M., Pittman, Q.J., and Veale, W.L. 1988. Stimulation of vasopressin release in the ventral septum of the rat brain suppresses prostaglandin E1 fever. J.Physiol. **399**: 177-189.

175. Naylor, A.M., Ruwe, W.D., Burnard, D.M., McNeely, P.D., Turner, S.L., Pittman, Q.J., and Veale, W.L. 1985a. Vasopressin-induced motor disturbances: localization of a sensitive forebrain site in the rat. Brain Res. **361**: 242-246.

176. Naylor, A.M., Ruwe, W.D., Kohut, A.F., and Veale, W.L. 1985b. Perfusion of vasopressin within the ventral septum of the rabbit suppresses endotoxin fever. Brain Res. Bull. **15**: 209-213.

177. O'Donohue, T.L., and Dorsa, D.M. 1982. The opiomelanotropinergic neuronal and endocrine systems. Peptides **3**: 353-395.

178. Ogata, K., and Murakami, N. 1972. Neural factors affecting the regulatory responses of body temperature. In: Advances in Climatic Physiology. S. Itoh, K. Ogata and H. Yoshimura, eds. Springer Verlag, New York. pp. 50-67.

179. Ottersen, O.P. 1980. Afferent connections to the amygdaloid complex of the rat and cat. II. Afferents from the hypothalamus and the basal telencephalon. J.Comp.Neurol. **194**: 267-289.

180. Ottersen, O.P. 1981. Afferent connections to the amygdaloid complex of the rat with some observations in the cat: III. Afferents from the lower brainstem. J.Comp.Neurol. **203**: 335-356.

181. Ottersen, O.P. 1982. Connections of the amygdala of the rat. IV. Corticoamygdaloid and intraamygdaloid connections as studied with axonal transport of horseradish peroxidase. J.Comp.Neurol. **205**: 30-48.

182. Ottersen, O.P., and Ben-Ari, Y. 1979. Afferent connections to the amygdaloid complex of the rat and cat. I. Projections from the thalamus. J.Comp.Neurol. **187**: 401-424.

183. Paxinos, G., and Watson, C. 1982. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.

184. Pearlmutter, A.F., Constantini, M.G., and Loeser, B. 1983. Characterization of 3H-AVP binding sites in particulate preparations of rat brain. J.Exp.Biol. **4**: 335-341.

185. Phillip-Dormstrom, W.K., and Siegert, R. 1974. Prostaglandins of the E and F series in rabbit cerebral spinal fluid during fever induced by Newcastle Disease virus, E. coli-endotoxin, or endogenous pyrogen. Med.Microbiol.Immunol. **159**: 279-284.

186. Pittman, Q.J., Blume, H.W., and Renaud, L.P. 1981. Connections of the hypothalamic paraventricular nucleus with the neurohypophysis, median eminence, amygdala, lateral septum and midbrain periaqueductal gray: an electrophysiological study in the rat. Brain Res. **215**: 15-28.

187. Pittman, Q.J., Malkinson, T.J., Kasting, N.W., and Veale, W.L. 1988. Enhanced fever following castration: possible involvement of brain arginine vasopressin. Am.J.Physiol. **254**: R513-R517.

188. Porter, J.P., and Brody, M.J. 1986. A V_1 vasopressin receptor antagonist has nonspecific neurodepressant actions in the spinal cord. Neuroend. **43**: 75-78.

189. Poulin, P., Lederis, K., and Pittman, Q.J. 1988. Subcellular localization and characterization of vasopressin binding sites in the ventral septal area, lateral septum, and hippocampus of the rat brain. J.Neurochem. **50**: 889-898.

190. Reitschel, E.T., Kim, Y.B., Watson, D.W., Galanos, C., Luderitz, O., and Westphal, O. 1973. Pyrogenicity and immunogenicity of lipid A complexed with bovine serum albumin. Infect.Immunol. **8**: 173-177.

191. Rezvani, A.H., Denbow, D.M., and Myers, R.D. 1986. α-Melanocyte-stimulating hormone infused icv fails to affect body temperature or endotoxin fever in the cat. Brain Res.Bull. **16**: 99-105.

192. Richards, D.B., and Lipton, J.M. 1984. Antipyretic doses of α-MSH do not alter afebrile body temperatures in the cold. J.Therm.Biol. **9(4)**: 299-301.

193. Rowley, D., Howard, J.G., and Jenkins, C.R. 1956. The fate of ^{32}P labelled bacterial lipopolysaccharide in laboratory animals. Lancet **i**: 366-367.

194. Ruwe, W.D., and Myers, R.D. 1979. Fever produced by intrahypothalamic pyrogen: Effect on protein synthesis inhibition by anisomycin. Brain Res.Bull. **4**: 741-745.

195. Ruwe, W.D., and Myers, R.D. 1980. The role of protein synthesis in the hypothalamic mechanism mediating pyrogen fever. Brain Res.Bull. **5**: 735-743.

196. Ruwe, W.D., Naylor, A.M., and Veale, W.L. 1985a. Perfusion of vasopressin within the rat brain suppresses prostaglandin E-hyperthermia. Brain Res. **338**: 219-224.

197. Ruwe, W.D., Naylor, A.M., and Veale, W.L. 1986. Neurohypophyseal peptides alter the characteristics of the febrile response in the cat. In: Homeostasis and thermal stress. 6th int. symp. pharmacol. thermoreg. jasper, alta. K.E. Cooper, P. Lomax, E. Schönbaum and W.L. Veale, eds. Karger, Basel. pp. 145-147.

198. Ruwe, W.D., Veale, W.L., Pittman, Q.J., Kasting, N.W., and Lederis, K. 1985b. Release of arginine vasopressin from the brain: correlation with physiological events. In: In Vivo Perfusion and Release of Neuroactive Substances. R. Drucker-Colin and A. Bayon, eds. Academic Press, New York. pp. 233-247.

199. Samson, W.K., Lipton, J.M., Zimmer, J.A., and Glyn, J.R. 1981. The effect of fever on central α-MSH concentrations in the rabbit. Peptides **2**: 419-423.

200. Saper, C.B., Swanson, L.W., and Cowan, W.M. 1976. The efferent connections of the ventromedial nucleus of the hypothalamus of the rat. J.Comp.Neurol. **169**: 409-442.

201. Satinoff, E., and Rustein, J. 1970. Behavioral thermoregulation in rats with anterior hypothalamic lesions. J.Comp.Physiol.Psychol. **71**: 77-82.

202. Satinoff, E., and Shan, S.Y.Y. 1971. Loss of behavioral thermoregulation after lateral hypothalamic lesions in rats. J.Comp.Physiol.Psychol. **77**: 302-312.

203. Sawchenko, P.E., and Swanson, L.W. 1983. The organisation of forebrain afferents to the paraventricular and supraoptic nuclei of the rat. J.Comp.Neurol. **218**: 121-144.

204. Sawyer, W.H., Acosta, M., and Manning, M. 1974. Structural changes in the arginine vasopressin molecule that prolong its antidiuretic action. Endocrin. **95**: 140-149.

205. Schoener, E.P., and Wang, S.C. 1975. Leukocyte pyrogen and sodium acetylsalicylate on hypothalamic neurons in the cat. Am.J.Physiol. **229**: 185-190.

206. Schwaber, J.S., Kapp, B.S., Higgins, G.A., and Rapp, P.R. 1982. Amygdaloid and basal forebrain direct connections with the nucleus of the solitary tract and the dorsal motor nucleus. Neurosci. **2(10)**: 1424-1438.

207. Shih, S.T., and Lipton, J.M. 1985. Intravenous α-MSH reduces fever in the squirrel monkey. Peptides **6**: 685-687.

208. Shih, S.T., Khorram, O., Lipton, J.M., and McCann, S.M. 1986. Central administration of α-MSH antiserum augments fever in the rabbit. Am.J.Physiol. **250(19)**: R803-R806.

209. Siegert, R., Phillip-Dormstrom, W.K., Radsak, K., and Menzel, H. 1976. Mechanisms of fever induction in rabbits. Infect.Immunol. **14**: 1130-1137.

210. Sigal, S.L. 1978. Fever theory in the seventeenth century: building toward a comprehensive physiology. Yale J.Biol.Med. **51**: 571-582.

211. Sofroniew, M.V. 1980. Projections from vasopressin, oxytocin, and neurophysin neurons to neural targets in the rat and human. J.Hist.Cytochem. **28(5)**: 475-478.

212. Sofroniew, M.V. 1983a. Morphology of vasopressin and oxytocin neurones and their central and vascular projections. In: The Neurohypophysis: Structure Function and Control, Progress in Brain Research, vol. 60. B.A. Cross and G. Leng, eds. Elsevier, New York. pp. 101-114.

213. Sofroniew, M.V. 1983b. Vasopressin and oxytocin in the mammalian brain and spinal cord. TINS **6**: 467-471.

214. Sofroniew, M.V., and Weindl, A. 1978a. Projections from the parvocellular vasopressin- and neurophysin-containing neurons of the suprachiasmatic nucleus. Am.J.Anat. **153**: 391-430.

215. Sofroniew, M.V., and Weindl, A. 1978b. Extrahypothalamic neurophysin-containing perikarya, fiber pathways and fiber clusters in the rat brain. Endocrin. **102**: 334-337.

216. Soma, L.R. 1971. Depth of general anesthesia. In: Textbook of Veterinary Anesthesia. L.R. Soma, ed. Williams & Wilkins, Baltimore, MD. pp. 178-187.

217. Sperk, G., Lassmann, H., Baran, H., Kish, S.J., Seitlelberger, F., and Hornykiewicz, O. 1983. Kainic acid induced seizures: neurochemical and histopathological changes. Neurosci. **10(4)**: 1301-1315.

218. Squires, R.D., and Jacobson, F.H. 1968. Chronic deficits of temperature regulation produced in cats by preoptic lesions. Am.J.Physiol. **214(3)**: 549-560.

219. Stitt, J.T. 1979. Fever versus hyperthermia. Fed.Proc. **38**: 39-43.

220. Stitt, J.T. 1980. The effect of cycloheximide on temperature regulation and fever production in the rabbit. In: Thermoregulatory Mechanisms and their Therapeutic Implications. B. Cox, P. Lomax, A.S. Milton and E. Schönbaum, eds. Karger, Basel. pp. 120-125.

221. Stitt, J.T. 1985. Evidence for the involvement of the organum vasculosum laminae terminalis in the febrile response of rabbits and rats. J.Physiol. **368**: 501-511.

222. Stitt, J.T. 1986. Prostaglandin E as the neural mediator of the febrile response. Yale J.Biol.Med. **59**: 137-149.

223. Stitt, J.T. 1990. Differential sensitivity in the sites of fever production by prostaglandin E within the hypothalamus of the rat. J.Physiol. (In Press)

224. Stitt, J.T., and Bernheim, H.A. 1985. Differences in endogenous pyrogen fevers induced by iv and icv routes in rabbits. J.Appl.Physiol. **59**: 342-347.

225. Stitt, J.T., and Hardy, J.D. 1975. Microelectrophoresis of PGE_1 onto single units in the rabbit hypothalamus. Am.J.Physiol. **229**: 240-245.

226. Stitt, J.T., and Shimada, S.G. 1985. The site of action of verapamil inhibition on the febrile responses of rats to endogenous pyrogen (EP). Fed.Proc. **44**: 438.(Abstract)

227. Stitt, J.T., and Shimada, S.G. 1989. Enhancement of the febrile responses of rats to endogenous pyrogen occurs within the OVLT region. J.Appl.Physiol. **67**: 1740-1746.

228. Stitt, J.T., Shimada, S.G., and Bernheim, H.A. 1984. Microinjection of zymosan and lipopolysaccharide into the organum vasculosum laminae terminalis of rats enhances their febrile responsiveness to endogenous pyrogens. In: Thermal Physiology. J.R.S. Hales, ed. Raven Press, New York. pp. 555-558.

229. Swanson, L.W. 1976. An autoradiographic study of the efferent connections of the preoptic region in the rat. J.Comp.Neurol. **167**: 227-256.

230. Swanson, L.W. 1977. Immunohistochemical evidence for a neurophysin-containing autonomic pathway arising in the paraventricular nucleus of the hypothalamus. Brain Res. **128**: 346-353.

231. Swanson, L.W., and Cowan, W.M. 1979. The connections of the septal region in the rat. J.Comp.Neurol. **186**: 621-656.

232. Takayama, K., Okada, J., and Miura, M. 1990. Evidence that neurons of the central amygdaloid nucleus directly project to the site concerned with circulatory and respiratory regulation in the ventrolateral nucleus of the cat: a WGA-HRP study. Neurosci.Lett. **109**: 241-246.

233. Thompson, R.H., Hammel, H.T., and Hardy, J.D. 1959. Calorimetric studies in temperature regulation: The influence of cold, neutral and warm environment upon pyrogenic fever in normal and hypothalectomized dogs. Fed.Proc. **18**: 159-159.(Abstract)

234. Tiberiis, B.E., McLennan, H., and Wilson, N. 1983. Neurohypophyseal peptides and the hippocampus. II. Excitation of rat hippocampal neurones by oxytocin and vasopressin applied in vitro. Neuropeptides. **4**: 73-86.

235. Tribollet, E., and Dreifuss, J.J. 1981. Localization of neurones projecting to the hypothalamic paraventricular nucleus area of the rat: a horseradish peroxidase study. Neurosci. **6**: 1315-1328.

236. Tribollet, E., Barberis, C., Jard, S., Dubois-Dauphin, M., and Dreifuss, J.J. 1988. Localization and pharmacological characterization of high affinity binding sites for vasopressin and oxytocin in the rat brain by light microscopic autoradiography. Brain Res. **442**: 105-118.

237. Trippodo, N.C., Jorgensen, J.H., Priano, L.L., and Traber, D.L. 1973. Cerebrospinal fluid levels of endotoxin during endotoxemia. Proc.Soc.Exp.Biol.Med. **143**: 932-937.

238. Valtin, H., Sawyer, W.H., and Sokol, H.W. 1965. Neurohypophyseal principles in rat homozygous and heterozygous for hypothalamic diabetes insipidus (Brattleboro strain). Endocrin. **77**: 701-706.

239. Van der Kooy, D., Loda, L.Y., McGinty, J.F., Gerfen, C.R., and Bloom, F.E. 1984. The organization of projections from the cortex, amygdala, and hypothalamus to the nucleus of the solitary tract in the rat. J.Comp.Neurol. **224**: 1-24.

240. van Leeuwen, F.W., and Caffé, A.R. 1983. Vasopressin-immunoreactive cell bodies in the bed nucleus of the stria terminalis of the rat. Cell Tissue Res. **228**: 525-534.

241. van Leeuwen, F.W., and Wolters, P. 1983. Light microscopic autoradiographic localization of [3H]arginine- vasopressin binding sites in the rat brain and kidney. Neurosci.Lett. **41**: 61-66.

242. Van Miert, A.S.J.A.M., Van Essen, J.A., and Tromp, G.A. 1972. The antipyretic effect of pyrozalone derivative and salicylates on fever induced with leukocytes or bacterial pyrogen. Arch.Int.Pharmacodyn. **197**: 388-391.

243. Vane, J.R. 1971. Inhibition of prostaglandin synthesis as a mechanism of action of aspirin-like drugs. Nature,New Biol. **231**: 232-235.

233. Thompson, R.H., Hammel, H.T., and Hardy, J.D. 1959. Calorimetric studies in temperature regulation: The influence of cold, neutral and warm environment upon pyrogenic fever in normal and hypothalectomized dogs. Fed.Proc. **18**: 159-159.(Abstract)

234. Tiberiis, B.E., McLennan, H., and Wilson, N. 1983. Neurohypophyseal peptides and the hippocampus. II. Excitation of rat hippocampal neurones by oxytocin and vasopressin applied in vitro. Neuropeptides. **4**: 73-86.

235. Tribollet, E., and Dreifuss, J.J. 1981. Localization of neurones projecting to the hypothalamic paraventricular nucleus area of the rat: a horseradish peroxidase study. Neurosci. **6**: 1315-1328.

236. Tribollet, E., Barberis, C., Jard, S., Dubois-Dauphin, M., and Dreifuss, J.J. 1988. Localization and pharmacological characterization of high affinity binding sites for vasopressin and oxytocin in the rat brain by light microscopic autoradiography. Brain Res. **442**: 105-118.

237. Trippodo, N.C., Jorgensen, J.H., Priano, L.L., and Traber, D.L. 1973. Cerebrospinal fluid levels of endotoxin during endotoxemia. Proc.Soc.Exp.Biol.Med. **143**: 932-937.

238. Valtin, H., Sawyer, W.H., and Sokol, H.W. 1965. Neurohypophyseal principles in rat homozygous and heterozygous for hypothalamic diabetes insipidus (Brattleboro strain). Endocrin. **77**: 701-706.

239. Van der Kooy, D., Loda, L.Y., McGinty, J.F., Gerfen, C.R., and Bloom, F.E. 1984. The organization of projections from the cortex, amygdala, and hypothalamus to the nucleus of the solitary tract in the rat. J.Comp.Neurol. **224**: 1-24.

240. van Leeuwen, F.W., and Caffé, A.R. 1983. Vasopressin-immunoreactive cell bodies in the bed nucleus of the stria terminalis of the rat. Cell Tissue Res. **228**: 525-534.

241. van Leeuwen, F.W., and Wolters, P. 1983. Light microscopic autoradiographic localization of [3H]arginine- vasopressin binding sites in the rat brain and kidney. Neurosci.Lett. **41**: 61-66.

242. Van Miert, A.S.J.A.M., Van Essen, J.A., and Tromp, G.A. 1972. The antipyretic effect of pyrozalone derivative and salicylates on fever induced with leukocytes or bacterial pyrogen. Arch.Int.Pharmacodyn. **197**: 388-391.

243. Vane, J.R. 1971. Inhibition of prostaglandin synthesis as a mechanism of action of aspirin-like drugs. Nature,New Biol. **231**: 232-235.

244. Veale, W.L., and Cooper, K.E. 1975. Comparison of sites of action of prostaglandin and leukocyte pyrogen in brain. In: Temperature Regulation and Drug Action. P. Lomax, E. Schönbaum and J. Jacob, eds. Karger, Basel. pp. 218-226.

245. Veale, W.L., and Whishaw, I.Q. 1976. Body temperature responses at different ambient temperatures following injections of prostaglandin E_1 and noradrenaline into the brain. Pharmacol.Biochem.Behav. **4**: 143-150.

246. Veening, J.G. 1978. Subcortical afferents of the amygdaloid complex in the rat: an HRP study. Neurosci.Lett. **8**: 197-202.
245. Villablanca, J., and Myers, R.D. 1965. Fever produced by microinjection of typhoid vaccine into hypothalamus of cats. Am.J.Physiol. **208**: 703-706.

247. Wasterlain, C.G., and Jonec, V. 1983. Chemical kindling by muscarinic amygdaloid stimulation in the rat. Brain Res. **271**: 311-323.

248. Weindl, A. 1969. Electron microscopic observations on the organum vasculosum of the lamina terminalis after intravenous injection of horseradish peroxidase. Neurology **19**: 295.(Abstract)

249. Weller, K.L., and Smith, D.A. 1982. Afferent connections to the bed nucleus of the stria terminalis. Brain Res. **232**: 255-270.

250. Wilkinson, M.F., and Kasting, N.W. 1986. Centrally applied vasopressin is antipyretic due to its effects on febrile set point. Proc. I.U.P.S. **XVI**: 119.07.(Abstract)

251. Wilkinson, M.F., and Kasting, N.W. 1990. Centrally acting vasopressin contributes to endotoxin tolerance. Am.J.Physiol. **258**: R443-R449.

252. Williams, J.W., Rudy, T.A., Yaksh, T.L., and Viswanathan, C.T. 1977. An extensive exploration of the rat brain for sites mediating prostaglandin-induced hyperthermia. Brain Res. **120**: 251-262.

253. Wit, A., and Wang, S.C. 1968. Temperature-sensitive neurons in the preoptic/anterior hypothalamic region: actions of pyrogens and acetyl salicylate. Am.J.Physiol. **215**: 1160-1169.

254. Wood, D.D., Bayne, E.K., Goldring, M.B., Gowen, M., Hamerman, D., Humes, J.L., Ihrie, E.J., Lipsky, P.E., and Staruch, M.J. 1985. The four biochemically distinct species of human interleukin 1 all exhibit similar biologic activities. J.Immunol. **134**: 895-903.

255. Woolf, C.J., Willies, G.H., Laburn, H.P., and Rosendorff, C. 1975. Pyrogen and prostaglandin fever in the rabbit. I. Effects of salicylate and the role of cyclic AMP. Neuropharm. **14**: 397-403.