THE UNIVERSITY OF CALGARY

# Central Vasopressin and Endogenous Antipyresis

by

Alasdair M. Naylor

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF MEDICAL SCIENCE

CALGARY, ALBERTA

JUNE, 1987

ISBN 0-315-38045-4

THE UNIVERSITY OF CALGARY

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Central Vasopressin and Endogenous Antipyresis", submitted by Alasdair M. Naylor in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Dr. W.L. Veale, Supervisor
Department of Medical Physiology

Dr. K.E. Cooper
Department of Medical Physiology

Dr. J.S. Davison
Department of Medical Physiology

Dr. J.E. Maloney
Department of Obstetrics & Gynaecology

Dr. R. Walker
Department of Biology

Dr. E. Zeisberger
External Examiner
Justus-Liebig University
Giessen, West Germany

June 1987.

ABSTRACT

Experiments were undertaken to investigate the hypothesis that vasopressin functions within the CNS as an endogenous antipyretic. In the rabbit, infusion of vasopressin into a lateral cerebral ventricle did not reduce endotoxin fever. However, push-pull perfusion of the VSA with vasopressin suppressed significantly the fever evoked by iv or icv endotoxin.

It is believed that prostaglandins of the E series are important in the genesis of fever. During perfusion of the VSA with a control solution, infusion of $PGE_2$ into a lateral ventricle evoked prompt hyperthermia. When vasopressin was present in the perfusion medium bathing the VSA the $PGE_2$ hyperthermia was attenuated. These data indicate that vasopressin suppressed the fever evoked by one putative mediator of fever.

Dependent upon the route and/or site of administration, vasopressin evoked a number of thermoregulatory actions in the rat. Infused icv, vasopressin produced short-lasting hypothermia. Injected into the POA, vasopressin elicited long lasting hyperthermia which was abolished by a $V_1$ receptor antagonist [$d(CH_2)_5$Tyr(Me)AVP]. Similar injections of vasopressin into the NA, VSA, SI or DMH were without effect on core temperature. Thus, vasopressin exerts a number of thermoregulatory actions that are both neuroanatomically and functionally specific.

The peptide specificity of vasopressin-induced antipyresis was tested using the neurohypophyseal peptide, oxytocin. Perfusion of vasopressin within the VSA of the cat induced a dose-related, site specific antipyresis. However, when oxytocin was perfused into these

sites, the febrile response was unaffected. Similarly, in the guinea-pig, vasopressin's antipyretic action also was site specific and not mimicked by oxytocin.

The central receptor mediating the antipyretic action of vasopressin was investigated using analogues directed against the peripheral subtypes of vasopressin receptor. Vasopressin suppressed the fever evoked by the central infusion of IL-1, an effect which was antagonized by the $V_1$ vasopressin antagonist, $d(CH_2)_5$Tyr(Me)AVP. In contrast to vasopressin, the $V_2$ receptor agonist, DDAVP, did not alter the time course or magnitude of IL-1 fever.

Since the antipyretic action of vasopressin was blocked by a $V_1$ vasopressin antagonist, this analogue was used to investigate the role of endogenous vasopressin in fever suppression. On injection into the VSA, the $V_1$ vasopressin antagonist enhanced IL-1 fever in a dose dependent manner. In contrast, a $V_2$ vasopressin antagonist [$d(CH_2)_5$D-ValVAVP], did not alter the time course of IL-1 fever. These data suggest that the antipyretic effect of vasopressin is mediated via an action on a $V_1$-like receptor and that endogenous vasopressin, released into the VSA, may be involved in limiting fever.

The mechanism of vasopressin induced antipyresis may involve a change in "set-point" rather than an inhibition of heat production/conservation since microinjection of vasopressin into antipyretic active sites in the VSA (in the absence of pyrogen) at a cool ambient temperature did not result in any fall in core temperature.

In conclusion, these findings support and strengthen the hypothesis that vasopressin may function within the brain as an endogenous antipyretic.

## ACKNOWLEDGEMENTS

My sincere thanks go to Dr. W.L. Veale and Dr. K.E. Cooper for their excellent supervision and for providing a pleasant environment in which to carry out my graduate work. I would like to thank Dr. J.S. Davison and Dr. J.E. Maloney for agreeing to serve on my Supervisory Committee and Dr. R. Walker for agreeing to serve on my Examination Committee. I thank Dr. Q.J. Pittman for serving on my candidacy exam committee and also for many helpful discussions. In addition, I would like to thank Dr. E. Zeisberger for reading this thesis and agreeing to serve as my external examiner. I would also like to thank Dr. W.D. Ruwe, Mr. L.G. Bauce and Mr. T.J. Malkinson for their expertise and technical advice. I acknowledge financial support from the Alberta Heritage Foundation for Medical Research. Finally, I would like to thank Mrs. Grace Olmstead for typing this thesis.

# DEDICATION

To Bob and Patricia.

TABLE OF CONTENTS

## LIST OF FIGURES

ABBREVIATIONS

| | |
|---|---|
| aCSF | Artificial cerebrospinal fluid |
| AH | Anterior hypothalamus |
| APR | Acute phase reaction |
| AVP | Arginine vasopressin |
| BST | Bed nucleus of the stria terminalis |
| °C | Degrees centrigrade |
| CNS | Central nervous system |
| CRP | C-reactive protein |
| CSF | Cerebrospinal fluid |
| DBB | Diagonal band of Broca |
| DDAVP | Desamino-D-arginine-8-vasopressin |
| DGAVP | Des-9-glycinamide-arginine-vasopressin |
| DMH | Dorsomedial hypothalamus |
| EP | Endogenous pyrogen |
| ga | Gauge |
| h | Hour |
| HPGP | Human pituitary glycopeptide |
| hpIL-1 | Human purified interleukin-1 |
| icv | Intracerebroventricular |
| IL-1 | Interleukin-1 |
| IL-2 | Interleukin-2 |
| im | Intramuscular |
| ip | Intraperitoneal |
| iv | Intravenous |
| kg | Kilogram |
| LAF | Lympocyte activating factor |

| | |
|---|---|
| LCV | Lateral cerebral ventricle |
| LE | Long-Evans |
| LEM | Leukocyte endogenous mediator |
| LS | Lateral septum |
| mg | Milligram |
| ml | Millilitre |
| mm | Millimetre |
| α-MSH | Alpha-melanotropin |
| NA | Nucleus accumbens |
| ng | nanogram |
| NK | Natural killer |
| nmole | Nanomole |
| n.s. | Not significant |
| NSAID | Non-steroidal anti-inflammatory drug |
| OVLT | Organum vasculosum lamina terminalis |
| OXY | Oxytocin |
| $PGE_1$ | Prostaglandin $E_1$ |
| $PGE_2$ | Prostaglandin $E_2$ |
| pI | Isoelectric point |
| pmole | Picomole |
| POA | Preoptic area |
| PO/AH | Preoptic anterior hypothalamus |
| PVN | Paraventricular nucleus |
| SAA | Serum amyloid A |
| SAE | Salmonella abortus equi |
| SCN | Suprachiasmatic nucleus |
| SEM | Standard error of the mean |

| | |
|---|---|
| SI | Substantia innominata |
| VMH | Ventromedial hypothalamus |
| VSA | Ventral septal area |
| YSI | Yellow Springs Instruments |
| µg | Microgram |
| µl | Microlitre |

# I. INTRODUCTION

## A. FEVER AND HOST DEFENCE

Fever is an elevation in body temperature which is regulated and defended by the host. Fever can be initiated by a wide number of agents including endotoxins, viruses, gram-positive bacteria, hypersensitivity and also by immune responses. A common process is believed to be responsible for the fever induced by these activators, namely the synthesis by various phagocytic cells of a pyrogenic material initially termed endogenous pyrogen (EP) but now known as interleukin-1 (IL-1) (see later and also Dinarello, 1984a for review). This mainly monocyte derived protein is then believed to interact with neurons near or within the central nervous system (CNS), perhaps via intermediaries, to evoke a rise in body temperature. In most instances, the rise in core temperature is a moderate one, seldom exceeding 3-4°C (Du Bois, 1949).

The regulated nature of febrile rises in body temperature distinguish fever from hyperthermia. Thus, the elevation in body temperature associated with febrile conditions is not just an increase in heat production or a decrease in heat loss, like in hyperthermia, but a coordinated rise in body temperature associated with thermoregulatory activity similar to hypothermic defence reactions. Fever is characterized, therefore, by an upward shift in the 'set point' for body temperature control (Liebermeister, 1871) and may be generated at any ambient temperature.

Fever is only one part of a complex series of events that occurs following infection or injury. The other components of the host response to infection include metabolic and immunologic modifications which are also due to an action of IL-1. Collectively, these alterations are referred to as the host defence response (Dinarello, 1984a). Whether the generation of fever is of any survival value is the subject of much debate. However, since fever has been retained phylogenetically (Kluger, 1986 for review), it is tempting to speculate that a febrile rise in body temperature affords some survival value to organisms that may respond to infection with fever. While moderate fevers may be beneficial, it has been shown that a point is reached when further elevations in core temperature may be detrimental to health (Banet, 1979; Kluger and Vaughn, 1978). Evidence exists now to suggest that an antipyretic substance, which is released within the brain of febrile animals, may regulate febrile changes in body temperature. This concept of "endogenous antipyresis" (Kasting et al., 1978; Kasting et al., 1979c; Kasting 1980; Veale et al., 1981), involving the neurohypophyseal peptide arginine vasopressin (AVP), is the main subject of this thesis.

In the following section, after a brief historical perspective, the actions of pyrogens will be discussed. The cell sources, synthesis, role and importance of IL-1 in the host defence response will be emphasized. Finally, the central mechanisms involved in fever generation and amelioration will be outlined with particular reference to recent developments.

## 1) Historical Aspects of Temperature Regulation

Descriptions of fever date back to the ancient Greeks who believed that the presence of fever during infection was a beneficial sign. However, although the survival value of fever is still in debate, many of the beliefs of the ancient Greeks regarding fever and its generation were put to rest in the 19th Century. The ancients, including Hippocrates and Galen believed that disease was caused when one or more of the four body humors (blood, phlegm, yellow bile and black bile) was produced in excess of the others. Fever was a direct consequence of this imbalance and once initiated, resulted in the excess humor being evacuated (see Sigal, 1978; Kluger, 1980).

It was not until the 18th and 19th Centuries that the theories regarding temperature regulation and fever were changed to those more recognizable today. The ability of the body to derive metabolic heat, rather than the concept of a "central fire" in the left ventricle of the heart, was postulated by Lavoisier (1777). In addition, the difference between homeothermy and poikilothermy was described (Hunter, 1778), but it was still unclear how a constant temperature could be maintained in homeothermic species.

With the hypothesis that a balance between heat production and heat loss existed, the regulated nature of temperature control was realized (Currie, 1798; Wunderlich, 1871). Further, Brodie demonstrated the importance of the CNS in the control of body temperature. In his classical experiments, he showed that when the neck vessels were ligated and the animal ventilated, body temperature fell despite the continued perfusion of the tissues with oxygenated blood (Brodie,

1811, 1812). In a continuation of these experiments, Chossat (1820) investigated the effects of sections made at various levels of the spinal cord. The higher the level of transection, the greater the cooling. Chossat concluded that sections made high in the neck caused a greater temperature drop because of the paralysis of a larger number of nerves. (In fact, Chossat surmised that the sympathetic nerves were involved). However, it was not until 1852 that Bernard demonstrated that heat loss was accomplished through nervous regulation of blood flow to the surface of the body by sympathetic nerves.

In 1876, Bernard introduced the theory of homeostasis which recognized the ability of the body to regulate its internal environment or "milieu interieur". In addition, Bernard agreed with Lavoisier on the importance of metabolic processes for heat production and suggested that constant body temperature was regulated by a dynamic balance between heat production and heat loss.

Concurrent with the changing views on the regulation of body temperature, the theories relating to fever were modified. Liebermeister (1871) was the first to postulate that fever was a regulation of body temperature at a higher level: "... while the healthy person musters up all means available to him to maintain his temperature at 37°, the feverish person does the same to maintain a temperature of, say, 40°". In addition, this German physician determined that fevers were often beneficial but detrimental if they were too high or persisted for too long (Liebermeister, 1887). Liebermeister considered also that the elevated temperature of feverish disease could be explained only in terms of a dysfunction within a central nervous regulatory mechanism. Similarly, Welch

(1888) and Richet (1898) deduced that an important "heat-regulating centre" in the brain was responsible for febrile temperatures. Furthermore, Welch hypothesized that a thermoregulatory centre existed in the brain which responded to bacterial pyrogens indirectly through an intermediary released from blood leukocytes, a postulate that is accurate today.

Although Brodie was one of the first to designate a role for the CNS in thermoregulation, the localization of such temperature regulating centres was carried out by other investigators. In some instances, lesions made in the brain (Tscheschichin, 1866; Naunyn and Quincke, 1869) were too crude and traumatic for any consistent conclusions to be deduced (Lomax, 1979). However, experiments where the corpus striatum was damaged (Ott, 1884; Richet, 1885; Aronsohn and Sachs, 1885) resulted in rises in body temperature. Similarly, lesions in the brainstem, corpus striatum and optic thalamus evoked an increase in core temperature which was accompanied by heat production. Further, direct application of heat or cold to the corpus striatum evoked a change in core temperature which was the opposite to that produced in the brain tissue (Barbour, 1921). Thus, at the turn of the 19th Century, it was the consensus that the corpus striatum was the most likely site for a temperature regulating centre.

The hypothalamus was considered an important thermoregulatory centre in the brain when it was shown that massive lesion of this structure abolished thermoregulation (Isenschmid and Schnitzler, 1914). However, the actual importance of the hypothalamus was realized when it was found that discrete lesions of select regions severely impaired thermoregulatory ability. Thus, lesions of the

anterior and posterior hypothalamus caused impaired thermoregulatory ability in hot and cold environments (Clark et al., 1939; Andersson et al., 1965; Carlisle, 1969). Lesions of the lateral hypothalamus selectively destroyed behavioural thermoregulatory responses (Satinoff and Shan, 1971) while ablation of the preoptic anterior hypothalamus selectively interfered with autonomic thermoregulatory mechanisms (Satinoff and Rustein, 1970). The importance of the preoptic anterior hypothalamus was demonstrated further when either electrical (Hemingway et al., 1954) or thermal (Beaton et al., 1941; Calvert and Findlay, 1975) stimulation of this structure resulted in thermoregulatory changes. In addition, the anterior hypothalamus has been linked to the action of pyrogens to produce fever (Bennett et al., 1957; Villablanca and Myers, 1965; Cooper et al., 1967; Jackson, 1967) and lesions of the posterior hypothalamus reduce the febrile response to pyrogens (Thompson et al., 1959; Cooper and Veale, 1974) and render animals poikilothermic (Clark et al., 1939).

It would appear from this information that the hypothalamic region is probably the most important thermoregulatory structure in the brain. However, in terms of fever responses, this may no longer be the case. Indeed, other nearby structures, e.g. the organum vasculosum of the lamina terminalis (OVLT) and septal area, along with hypothalamic nuclei, may play vital roles in the development and amelioration of the febrile response.

2) Exogenous Pyrogens

Exogenous pyrogens are those substances which exist outside the body and which are pyrogenic following administration to human or experimental animals. The sources of exogenous pyrogens are diverse, including microorganisms, microbial products, inflammatory agents, antigens, plant lectins, lymphokines and some drugs. The fever producing properties of the above activators are a result of the synthesis and release of IL-1 (Dinarello, 1984b). The most potent (and most investigated) exogenous pyrogens are bacterial endotoxins derived from gram negative bacteria such as Escherichia, Salmonella and Shigella.

Endotoxin is composed of lipopolysaccharides which themselves consist of three identifiable units. These are the O-specific polysaccharide, the core polysaccharide and the lipid A fraction (Luderitz et al., 1971). It is the lipid moiety which is responsible for most of the toxic and biologic activities of endotoxin. Direct evidence for the pyrogenic action of lipid A has been provided by a number of investigators (Galanos et al., 1972; Rietschel et al., 1973), but its full biological activity is apparent only when it is associated chemically with the polysaccharide portion of the molecule (Kennedi et al., 1982).

Although it has been reported that endotoxin-like pyrogen has been found within the CNS following peripheral administration (Bennett et al., 1957), it has never been demonstrated conclusively that pyrogens of any kind can cross the blood brain barrier. Of all experiments involving bolus injections of $^{32}$P-endotoxin (Rowley et al,

1956) $^{51}$Cr-endotoxin (Braude et al., 1955) and $^{131}$I-endotoxin (Cooper and Cranston, 1963) into the circulation or continuous intracarotid infusion of $^{51}$Cr-endotoxin (Dascombe and Milton, 1979), all have failed to provide any evidence that peripherally administered endotoxin enters brain tissue. Similarly, analysis of endotoxin levels in cerebrospinal fluid (CSF), using the Limulus assay, revealed no endotoxin in the brain following systemic injection of relatively high doses (Trippodo et al., 1973). It must be concluded, therefore, that the febrile actions of peripheral endotoxin are attributable to an effect outside the CNS, a point which is not surprising in view of the large molecular weight of these substances. The actions of exogenous pyrogens on the reticuloendothelial system will be discussed in the next section.

3) Interleukin-1

a) Background

In response to microbial infection, tissue injury, immunologic and inflammatory disease, the host organism effects a dramatic change in internal homeostasis. Current evidence implicates the host rather than the invading microorganism in the mediation of the host-defence response. Early evidence supporting such a concept was provided by Beeson who demonstrated that fever was caused by a soluble leukocyte product which he named granulocyte pyrogen (Beeson, 1948) as its source was believed to be granulocytes. Subsequently, a protein with similar physical properties was observed in the blood of febrile rabbits (Grant and Whalen, 1953; Atkins and Wood, 1955) and humans

(Gerbrandy et al., 1954); this protein was termed endogenous pyrogen. In addition to endogenous pyrogen, a host phagocyte product which mediated the protein and divalent cation altering effects observed during infection, was isolated and termed leukocytic endogenous mediator (Kampschmidt et al., 1973 for review). Finally, immunologists were investigating the activation of T lymphocytes by a factor believed to originate in mononuclear phagocytes. The initial characterization of the soluble substance, named lymphocyte activating factor, was its ability to promote and augment thymocyte proliferation in response to the plant lectins phytohemagglutinin and concanavalin A (the LAF assay) (Gery et al., 1972). These three activites have been attributed to a phagocyte product or family of products now termed interleukin-1.

b) Interleukin Nomenclature

The term "interleukin-1" is a relatively recent addition to the field of experimental biology and therefore requires some definition. The name interleukin-1 (messenger between leukocytes) was adopted in 1979 to account for the biological actions of the phagocyte derived products - lymphocyte activating factor (LAF), leukocyte endogenous mediator (LEM) and endogenous pyrogen (EP) (see Aarden et al., 1979). These soluble protein factors are responsible for the immunological, metabolic and febrile components respectively of the host defence response (Gery, et al., 1972; Kampschmidt, 1981 for review; Beeson, 1948; Dinarello and Wolff, 1982 for review).

One of the reasons for the introduction of such nomenclature was that evidence existed to suggest that the actions of LAF, LEM and EP

were due to a common mediator. Thus, it was demonstrated that during and after purification (5 steps), the dose-response relationship between neutrophilia, the decrease in plasma levels of iron and zinc and the induction of a 1°C fever in rabbits was constant (Merriman et al., 1977; Rosenwasser and Dinarello, 1981). In addition, the interleukin nomenclature was devised to distinguish other soluble products that were potent immunoregulatory molecules, e.g. interleukin-2 (IL-2).

c) Cell Sources and Production of Interleukin-1

The cells important for IL-1 production are the mononuclear phagocytes since they are the most potent sources (Dinarello, 1984a). These include monocytes in blood, fixed macrophages in the blood, lung, 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; Gander and Goodale, 1975; Dinarello, 1984a for review). Of particular interest is the finding that glial cells are capable of producing IL-1 (Fontana et al., 1982) and IL-1 synthesis is induced in the brain of mice following peripheral treatment with endotoxin (Fontana et al., 1984). The wide distribution of cell types capable of producing IL-1 and their abundance at strategic primary defence locations is supportive of a role for IL-1 in the activation of the host defence response.

Among the most potent inducers of IL-1 production are bacteria and microbial products requiring only 5 ng/kg to induce fever and other aspects of the acute phase reaction (APR: see later) (Kampschmidt, 1981). The exact mechanism whereby bacteria stimulate

IL-1 synthesis is not known although a Ia surface antigen on mononuclear phagocytes has been implicated (Gilman et al., 1983). One possibility is that an inducible protein requiring RNA and protein synthesis is involved (Oppenheim et al., 1982) since actinomycin and puromycin can block IL-1 production in vitro. Although endotoxins can cause membrane perturbation changes, it is unlikely that this is the stimulus for IL-1 production since other agents that affect cell membranes, e.g. tuftsin, do not induce IL-1 production from human monocytes (Lachman, 1983). Thus, since there is little evidence for preformed stores of IL-1 (Fessler et al., 1961), it is generally considered that IL-1 is an inducible substance.

Regulation of IL-1 synthesis by prostaglandins has been investigated extensively since they are released simultaneously with IL-1 from mononuclear phagocytes. Inhibition of cyclooxygenase does not reduce IL-1 release (Hoo et al., 1972) and may, in fact, enhance it (Parant et al., 1980; Kunkel et al., 1986). In contrast, inhibition of the lipoxygenase pathway has been shown to inhibit IL-1 production indicating that a product of arachidonate lipoxygenase may be important in the sequence of events underlying cell activation for the production of IL-1 (Dinarello et al., 1983).

The presence of IL-1 also has been demonstrated in vivo in the plasma of humans (Dinarello and Wolff, 1979; Hellon and Townsend, 1983 for reviews) and animals during experimental fever. However, difficulties demonstrating its presence during specific disease states have arisen because of "suppressive factors" which appear to mask the action of IL-1 in the various assay systems. One of these suppressive factors may be a 20,000-40,000 M.W. protein isolated from the urine of

febrile patients (Liao et al., 1984). Other evidence for the presence of IL-1 in vivo is that the monokine is found in the plasma of menstruating women. This suggests that IL-1 may have a role in normal physiology since the levels vary with the menstrual cycle (Cannon and Dinarello, 1985), also, spontaneous production of IL-1 by human Hodgkin's tissue may explain the long and sustained fevers observed in patients with certain cancers.

d) Role of Interleukin-1 in the Host Defence Response

Release of IL-1 by cells of the reticuloendothelial system is believed to initiate a series of events that benefit the host in combating invading microorganisms (or other foreign factors). These include fever, metabolic changes, as well as general stimulation of the immune system and are referred to collectively as the acute phase response (APR). A prominent feature of the APR is the generalized nature of the reaction, such that local injury or complete systemic assault will stimulate the host defence mechanisms.

i) Fever

Fever is the most prominent feature of infection since it is the most commonly observed clinical sign of disease. However, the benefits of fever to the host are the subject of much debate (Kluger, 1986; Dinarello et al., 1986; Blatteis, 1986, Banet, 1986; Duff, 1986). While Lizard (Vaughn et al., 1974; Kluger et al., 1975) and fish (Covert and Reynolds, 1977) survival is enhanced significantly at higher temperatures following pyrogen administration, in rabbits (Kluger and Vaughn, 1978) and rats (Banet, 1979), the optimum effect on survival occurs only at moderate febrile levels. Indeed, there is

a point at which any further rise in core temperature may be detrimental to the health of the animal. In contrast, physical cooling of the abdominal vena cavae of rabbits following an intravenous (iv) injection of live Pasteurella multocida (which prevents the rabbit attaining febrile body temperature) increased the survival rate from 46% in febrile rabbits to 90% in the cooled rabbits (Vaughn, Veale and Cooper, unpublished observations). In addition, some fish (Marx et al., 1984) and lizards (Laburn et al., 1981) do not respond to endotoxin, PGE or crude IL-1 with fever. Consequently, there is still much confusion surrounding both the potential benefits and uniformity of fever.

It may be that the elevation in temperature per se is not the only important aspect here but rather how febrile rises in core temperature interact with other components of the APR to combat invading microorganisms. For example, febrile rises in body temperature decrease the ability of microorganisms to produce iron transport compounds, thereby reducing the availability of iron for bacterial growth (Weinberg, 1978). The importance, and deleterious effect on bacteria, of such an interaction has been demonstrated in vitro (Kluger and Rothenberg, 1979). In addition, the direct effect of temperature on cells responsible for host defence functions, e.g. macrophages, neutrophils and lymphocytes may be relevant. For example, hyperthermia: 1) augments the proliferative activity of IL-1 in cell culture systems (Duff and Durum, 1983); 2) increases the production and killing efficiency of cytoxic T lymphocytes (Dinarello et al., 1986) and 3) augments antibody production. In apparent contrast, febrile temperatures decrease the activity of natural killer

(Nk) cells (Dinarello et al., 1986). However, caution should be exercised when attempting to extrapolate information obtained in vitro to what actually takes place in vivo.

On a more practical level, elevated temperatures retard the growth of certain tumors (Lih and Hahn, 1984) and pyrogenic endotoxins (e.g. Coley's toxins) have been reported to be beneficial in the treatment of cancer (Coley, 1919; Miller and Nicholson, 1971). However, whether this is a direct consequence of increased temperature, or whether stimulation of other aspects of the APR are responsible is not clear at present.

ii) Effects on plasma divalent cations

Another host defence role ascribed to IL-1 (and a characteristic of infection) is the reduction in the plasma levels of iron and zinc and the elevated plasma levels of copper (Kampschmidt, 1981). Iron is important for bacterial growth (Kluger and Rothenburg, 1979) and zinc is required for incorporation into bacterial metalloenzymes and for membrane stabilization (Sugarman, 1983 for review). Reducing the plasma levels of these ions is therefore clearly beneficial to the host. However, during prolonged periods of infection, anaemia, zinc dependent enzyme dysfunction and depressed T cell function may result. Copper is important for the immune system, particularly the antibody producing B cells which may be reduced in number during marginal copper deficiency (Prohaska and Lukasewycz, 1981). The increased levels of copper are due to hepatic synthesis of ceruloplasmin, a serum component which may also fulfill an anti-inflammatory role by picking up toxic superoxide anion radicals (Goldstein et al., 1982).

iii) Metabolic alterations

The APR is associated also with a marked increase in the synthesis of hepatic protein - the so called "acute phase reactants" which include C-reative protein (CRP), serum amyloid A protein (SAA), haptoglobulin, fibrinogen, $\alpha_1$ macroglobulin and ceruloplasmin. The precise functions of all these proteins are not fully understood. It has been suggested that CRP and SAA may serve some immunoregulatory function. In this regard, SAA suppresses antibody responses in vitro (Benson et al., 1975) and CRP enhances phagocytosis (Mold et al., 1982). Further, CRP may promote healing by binding to necrotic cells at sites of injury (Kushner and Kaplan, 1961). Likewise, fibrinogen is most likely concerned with tissue injury and healing.

The source of amino acids for the synthesis of hepatic proteins is from muscle, a process which also is mediated by IL-1 (Baracos et al., 1983). During periods of prolonged infection, these muscle derived amino acids are oxidized for energy (Wannemacher, 1977), a process which leads to increased urea excretion, loss of body weight and net nitrogen catabolism. This proteolytic action of IL-1 may not be beneficial to the host since it probably occurs when food intake is reduced as IL-1 depresses appetite (McCarthy et al., 1985) and induces sleep (Kreuger et al., 1984).

iv) Immune system

The effects of IL-1 on the immune system may be the most important with respect to the host-defence response. Once in the circulation, IL-1 stimulates the bone to release neutrophils (Kampschmidt, 1984 - for review). Interleukin-1 also appears to act as a maturational signal, preparing T cells to respond to antigens or

secondary mediator signals (Mizel, 1982). Interleukin-1 also stimulates lymphocytes to initiate cellular and humoral immune mechanisms. Thus, IL-1 has been implicated in B-cell proliferation and antibody production (Lipsky et al., 1983). Perhaps the major role of IL-1 is to induce the synthesis of the T-cell derived mitogenic lymphokine, IL-2 (Smith et al., 1980). The link between IL-1 and IL-2 is that IL-1 signals the T cells to produce IL-2 which in turn results in T cell proliferation, and, in conjunction with interferon alpha enhances natural killer cell activity (Dempsey et al., 1982).

v) Conclusions

In conclusion, IL-1 is an important component of the host defence response which is released in response to any kind of assault. It is responsible for a number of coordinated events which, when grouped together, are considered detrimental to invading microorganisms. Further, IL-1 represents a link between the host-response to injury and the immune system.

e) Sequence Analysis and Heterogeneity of Interleukin-1

One of the current hypotheses is that all of the biological and diverse actions discussed in the previous section are mediated by a single monokine, IL-1. However, it has been known for some time that IL-1 has a large degree of charge heterogeneity, as shown by three or more isoelectric points (pI), which are related to molecular weight differences (Wood et al., 1985). Therefore, it would appear that IL-1 exists as a family of polypeptides. The cloning of murine pI 5 (Lomedico et al., 1984) and human pI 7 IL-1 (Auron et al., 1984) has demonstrated that these two IL-1's are derived from two distinct gene

products. In addition, there appears to be two identifiable IL-1's (i.e. different gene products) from human monocytes (Auron et al., 1985) with the cDNA from the pI 7 form coding for approximately 25 percent amino acid homology with cDNA from the pI 5 form. New molecular species of IL-1 (pI 5.5) have been described which have many of the activities of human pI 7 monocytic IL-1 (Rimsky et al., 1986; Knudsen et al., 1986) but which may represent structurally unique IL-1 species as determined by sequence analysis, size and antibody reactivity.

Nomenclature recently has been agreed upon for the description of recombinant IL-1. The acidic (pI 5) form being designated as $IL\text{-}1_{\alpha}$ and the neutral (pI 7) form as $IL\text{-}1_{\beta}$ (Oppenheim, 1986). The small amount of homology between these two is observed at the carboxyterminus which may represent the active site of the molecule. However, it has not been possible to ascribe a particular biological activity to any one species of IL-1. For this, it will be necessary to purify each IL-1 to homogeneity and characterize it in a number of in vivo and in vitro assay systems. This task is compounded further by the demonstration that some of the biologic activities of IL-1 are conserved in small molecular weight fragments which have been isolated from the plasma of febrile patients (Dinarello et al., 1984).

4) Central Mechanisms Involved in Fever Generation

In a previous section, the ability of exogenous pyrogens to evoke fever was discussed in relation to their ability to induce the synthesis of IL-1 from cells of the reticuloendothelial system.

Further, it has been documented how important the CNS is in the drive to febrile temperatures. This section will attempt to explain the mechanisms involved in fever generation by examining 1) the concept of "set-point"; 2) the actions of pyrogens on neurons within (or close to) the brain; 3) possible site of entry of IL-1 into the CNS; and 4) the actions of neurochemicals, released within the brain, which may be the ultimate mediators of febrile rises in body temperature.

a) Set Point Theory

The ability of mammals to maintain a constant body temperature over a wide range of thermal conditions suggests that an internally regulated reference or set point for temperature regulation exists. Although such a concept may explain how body temperature remains constant, little is known about the mechanisms underlying it. A change in the set point around which body temperature is regulated has long been utilized to explain the elevation in temperature observed during fever. Thus, Liebermeister (1871) proposed that the set point is elevated during fever, a concept that has been supported by many investigators (Barbour, 1921; Du Bois, 1936). In addition, the body will defend actively against thermal challenges during fever (Macpherson, 1959; Cooper et al., 1964), demonstrating the regulated nature of febrile rises in body temperature. Thus, the magnitude of fever produced by a given amount of pyrogen remains constant over a broad range of ambient temperatures. In the cold, fever is produced by a large increase in heat production, while in the heat, febrile temperatures may be attained by decreasing heat loss (Stitt, 1979). Additional evidence supporting the elevation of set point during fever

is that, following pyrogen administration, dogs (Cabanac et al., 1970), cats (Weiss, et al., 1967) and newborn rabbits (Satinoff et al., 1976) select a warmer ambient temperature than when they were afebrile.

i) Ionic theory

While it is generally believed that body temperature is regulated around a higher level during fever, the actual central mechanisms involved in such an alteration are not understood. However, a potential mechanism for a set point alteration has been described (Myers and Veale, 1970, 1971). In this theory, it was proposed that the ratio of $[Na^{+}]$ to $[Ca^{++}]$ in the extracellular fluid of the posterior hypothalamus was responsible for determining the set point about which body temperature was regulated. This "ionic theory" was based on the observations that excess $Ca^{2+}$ caused animals to regulate at a lower body temperature whereas excess $Na^{+}$ caused animals to regulate at febrile body temperatures. Evidence supporting a role for the "ionic theory" in the raised set-point observed during fever was provided by Myers and Tytell (1972) who demonstrated that the efflux of $^{45}Ca^{2+}$ into the third ventricle was increased, whereas that of $^{22}Na^{+}$ was decreased during the rising phase of fever. In agreement with the hypothesis, increased amounts of $^{22}Na^{+}$ were detected in the ventricle and $^{45}Ca^{2}$ efflux was reduced during defervescence. How an altered ratio of ions within the posterior hypothalamus might interact with thermoregulatory centres in the anterior hypothalamus to activate thermoregulatory effector pathways is not known but may be via their neural connections (Adair, 1974). However, the posterior hypothalamus appears to be important in fever generation since ablation of this

structure abolishes febrile rises in body temperature (Thompson et al., 1959; Cooper and Veale, 1974).

b) Effects of Pyrogens on Central Neuronal Activity

In contrast to the theory of set point and its elevation during fever are electrophysiological studies which have examined the activity and thermoresponsiveness of single units within the anterior hypothalamus and septum following intravenous or localized administration of pyrogens (See Eisenman, 1982 for review). The following sections will outline the evidence supporting the hypothesis that febrile temperatures are brought about by a direct action of pyrogens on hypothalamic neurons.

i) Bacterial and endogenous pyrogen (interleukin-1)

Following systemic injection of bacterial (Cabanac et al., 1968; Wit and Wang, 1968; Eisenman, 1969, 1974) or endogenous (Schoener and Wang, 1975; Belyavskii and Abramova, 1975) pyrogen, the activity and thermosensitivity of PO/AH warm sensitive neurons was decreased, whereas PO/AH cold sensitive neurons showed increased spontaneous activity and firing. These actions, which are reversed by antipyretics (Wit and Wang, 1968; Schoener and Wang, 1975), are those anticipated for increased heat storage which would lead to febrile body temperatures (Eisenman, 1982). It was suggested, therefore, that pyrogens might induce fever by a reduction in thermosensitivity rather than a displacement of set point (Mitchell et al., 1970; Eisenman, 1974). However, the physiological significance of this depressed thermosensitivity is not clear since most studies on thermoregulation during fever showed no decrease in thermoregulatory ability

(Macpherson, 1959; Cooper et al., 1964). Furthermore, intrahypothalamic administration of prostaglandin $E_1$ ($PGE_1$) did not alter anterior hypothalamic thermosensitivity to thermode heating (Stitt and Hardy, 1975). It has been found in other studies, however, that a decrease in an animal's ability to respond to PO/AH thermal stimulation exists following pyrogen administration (Eisenman, 1974; Lipton and Kennedy, 1979).

In support of the hypothesis that these pyrogens influenced PO/AH neurons directly, microinjection of crude IL-1 into the PO/AH, close to the recording electrode, produced actions with a rapid onset similar to those observed following peripheral administration of pyrogens (Schoener and Wang, 1975). A concern with the direct intracerebral administration of pyrogens is that there is no evidence that these substances can enter brain tissue (Rowley et al., 1956; Braude et al., 1955; Cooper and Cranston, 1963; Dascombe and Milton, 1979; Trippodo et al., 1973; Dinarello et al., 1979), even though injection of pyrogens into the brain evokes fever (Sheth and Borison, 1960; Villablanca and Myers, 1965; Cooper et al., 1967; Jackson, 1967). However, despite the fact that endotoxin does not cross the blood brain barrier, the presence of this substance in the periphery may induce the central synthesis of IL-1 from glial cells (Fontana et al., 1982; Fontana et al., 1984), thereby precluding the requirement for IL-1 to cross into the brain from the periphery.

ii) Prostaglandins

With the discovery of the pyrogenic action of prostaglandins of the E series (Milton and Wendlandt, 1971) and the demonstration of the antipyretic properties of inhibitors of prostaglandin synthesis, e.g.

aspirin (Vane, 1971), the concept developed that fevers resulted from the synthesis and release of PGE at sensitive brain sites (see later). Therefore, the effects of PGE on neuronal thermosensitivity and firing was examined. In contrast to the data obtained using bacterial or endogenous pyrogen, electrophysiological studies with prostaglandins have not produced clear results (Eisenman, 1982 for review). In an extensive study it was found that 90% of cells tested were unresponsive to $PGE_1$ regardless of local thermosensitivity (Stitt and Hardy, 1975). In another study, the response to application of PGE could not be correlated to physiological thermal input (Jell and Sweatman, 1977). However, other less extensive investigations have revealed data consistent with a direct pyrogenic action of PGE on PO/AH thermosensitive neurons (Ford, 1974; Schoener and Wang, 1976; Gordon and Heath, 1979).

At present, single unit studies do not support a role for PGE in fever. Furthermore, it seems that the control of body temperature and the generation of fever are unlikely to be determined by altered central thermosensitivity alone. In addition, reports on PO/AH thermosensitivity during desynchronized sleep suggests that it may be lost under these conditions (Azzarone et al., 1985). This factor will make the interpretation of the data more confusing since a number of preparations used to investigate single unit activity require an anaesthetic. This could possibly account for the observed discrepancy between the effects of pyrogens and PGE on thermosensitive neurons.

c) Proposed Central Mechanism of Action of Interleukin-1

It is generally accepted that the synthesis and release of IL-1 is the first step in the generation of fever. Interleukin-1 is then carried in the blood to the brain, via the cerebral circulation, where it interacts at or near the PO/AH. That crude IL-1 can evoke fever when injected into the PO/AH has been demonstrated by a number of investigators (Cooper et al., 1967; Jackson, 1967), though it is possible that intermediaries such as prostaglandins may be involved also. Such an interaction of pyrogens within the PO/AH is then thought to initiate the changes in thermoregulatory effectors which produce fever. Although this hypothesis has long been used to describe the events leading up to fever, information regarding the interaction of IL-1 with a site at or near the brain has always been missing. Recent evidence implicates a circumventricular organ, the OVLT, as the central target for circulating IL-1.

i) Site of action

The OVLT is a portion of the brain located in the rostral wall of the third ventricle adjacent to the PO/AH and medial septum but outside the blood brain barrier (Wiendl, 1969). This location, coupled with the absence of tight-junctioned vascular epithelial cells, enables larger protein molecules to interact closely with the perivascular space of the OVLT. The close proximity of the OVLT to pyrogen sensitive areas in the PO/AH makes this structure a possible portal of entry for IL-1 to brain tissue.

The first evidence implicating the OVLT in such a role was provided by Blatteis and co-workers (1983) who reported that lesions of the anteroventral portion of the third ventricle abolished

endotoxin-induced fevers in guinea-pigs. Later, in apparent contrast, it was observed that discrete lesions placed in the OVLT augmented and enhanced the sensitivity of rats and rabbits to systemically administered crude IL-1 (Stitt, 1985). This augmented febrile response following the lesion gradually returned towards normal within 3 weeks. The differences between the two studies were attributed to the extent of lesioning, which was much more pronounced in the former study. In addition, Stitt postulated that the IL-1 was acting within the OVLT at some kind of reticuloendothelial cell which was sensitized following lesioning, perhaps as a result of some irritative or inflammatory reaction (Stitt, 1985; Stitt, 1986). In support of this, microinjection of immuno-adjuvants such as zymosan, directly into the OVLT, resulted in enhanced fevers similar to those observed following lesion of the OVLT (Stitt et al., 1984). In accordance with the hypothesis, mesenchymally derived phagocytic cells are present within the perivascular space of the OVLT (Murabe et al., 1981).

The hypothesis that IL-1 might interact with some receptor or mesenchymal cell in the OVLT is attractive for a number of reasons. Firstly, it explains why EP has never been demonstrated to cross the blood brain barrier (Dinarello et al., 1978) and, secondly, it may account for the shorter latency observed following intravenous versus intracerebroventricular administration of pyrogens since the target would be closer to the circulation rather than the brain tissue (Stitt, 1986).

ii) Role of $Ca^{++}$ channels

The host produces IL-1 as a mediator of the acute phase response. An important question is what are the molecular mechanisms involved in the action of IL-1, particularly as its biological actions are observed so quickly following administration. Dinarello (1984a) has proposed that the actions of IL-1 can be best explained in terms of a calcium ionophore. This is because biological actions attributed to IL-1 are mimicked by the calcium ionophore A23187. For example, A23187 increases the synthesis of PGE (Betteridge, 1980) is mitogenic for T cells (Luckasen et al., 1974), promotes neutrophil degranulation (Klempner et al., 1978) and substitutes for IL-1 in the LAF assay (Koretzky et al., 1983). Presumably, the resultant increase in intracellular calcium activates the second messenger.

In the pathways to fever generation, two enzymes may be involved in the production of PGE, namely phospholipase $A_2$ and cyclooxygenase, which are responsible for the production of arachidonic acid and PGE respectively. Since phospholipase $A_2$ is activated by $Ca^{++}$ and in view of the calcium ionophore A23187 substituting for IL-1, the role of $Ca^{++}$ channels in the febrile effect of IL-1 was investigated. Either intravenous injection or direct microinjection of the calcium channel blocker, verapamil, into the OVLT blocked IL-1 (injected iv) but not PGE (injected into the OVLT) induced fever, suggesting that the febrile action of IL-1 required an influx of $Ca^{++}$ (Stitt and Shimada, 1985). These data not only suggest that calcium channels are involved in the febrile action of IL-1 but also strengthen the hypothesis that the OVLT is important in the transduction of the febrile signal from

IL-1 to the brain. The importance of the activation of phospholipase $A_2$ within the OVLT will be discussed later.

iii) Central synthesis of interleukin-1

There is concern that the central injection of IL-1 into the ventricular system of the brain represents an artificial situation since IL-1 may not enter the CNS (Dinarello et al., 1978) and the characteristics of iv and icv fevers are different (Stitt and Bernheim, 1985). However, if cells within the brain were to synthesize IL-1 then this might provide a case where the central injection of pyrogens was relevant physiologically.

It has been demonstrated that cultured astrocytes and glioma cells can synthesize IL-1 (Fontana et al., 1982). Furthermore, it has been reported that IL-1 synthesis is induced in the brain of mice after peripheral treatment with endotoxin (Fontana et al., 1984). Since endotoxin and IL-1 may not cross the blood brain barrier, some other factor must be responsible for the centrally synthesized IL-1 observed under these conditions. Moreover, it suggests that central actions of IL-1 may be physiologically relevant and that the CNS, particularly glial cells, may possess some immunoregulatory function (Stein et al., 1976; Blatteis et al., 1984.

d) Central Mediators of Fever

The thermal component of fever is a result of an interaction between circulating IL-1 and the CNS. Since circulating IL-1 may not enter the brain tissue, central mediators of fever have been sought

which might be responsible for febrile rises in body temperature. This section will outline the evidence implicating mediator substances in the drive to febrile body temperatures.

i) Role of protein mediators

Evidence exists to support the hypothesis that protein synthesis is important for the generation of fever. Systemic treatment of rabbits with cycloheximide prevented the febrile actions of both endotoxin (Siegert et al., 1976) and endogenous pyrogen (Siegert et al., 1976; Cranston et al., 1978). However, cycloheximide has a number of other effects on body processes, including an incapacitating effect on thermoregulation (Barney et al., 1979; Stitt, 1980) and so the relevance of experiments using this relatively toxic drug are in question.

Another inhibitor of protein synthesis, anisomycin (Grollman and Walsh, 1967), has been used to investigate the role of protein mediators in fever generation. This inhibitor can block the fever induced by either peripherally or centrally administered pyrogens (Ruwe and Myers, 1979; Ruwe and Myers, 1980; Cranston et al., 1980; Cranston et al., 1982). Furthermore, microinjection of anisomycin directly into the PO/AH can delay or prevent endotoxin induced fever suggesting that de novo synthesis of protein, within this area of the brain, is important for the development of fever (Ruwe and Myers, 1980). Unlike cycloheximide, anisomycin does not compromise the thermoregulatory capacity of the animals (Cranston et al., 1980; Ruwe and Myers, 1980), suggesting that a non-specific action of the inhibitor is unlikely to be responsible for the antipyretic effects of the drug. Evidence demonstrating that anisomycin inhibits

hypothalamic protein synthesis, rather than some other pharmacological action, has been provided by Cranston et al., (1982).

The actual mechanisms involved in the fever reducing properties of drugs like anisomycin may involve an interference with the arachidonic acid cascade whereby prostaglandins are produced. Thus, both fever and the increase in CSF levels of $PGE_2$ normally observed following pyrogen injection were prevented by anisomycin. This may be because anisomycin inhibits the formation of phospholipase $A_2$ (Townsend et al., 1984) since preventing the action of this enzyme abolishes fever (Cranston et al., 1983). Experiments carried out in vitro also have shown that inhibitors of protein synthesis prevent the elaboration of $PGE_2$ from hypothalamic tissue (Bernheim and Dinarello, 1985). While inhibition of PGE synthesis might explain the reduction in pyrogen fever observed following protein synthesis inhibition it cannot account for the observation that anisomycin blocks PGE hyperthermia (Milton and Sawhney, 1981; Ruwe and Myers, 1980). These data suggest that anisomycin may be acting at a site after PGE in the pathway to fever. In support of this, and in contrast to the work described above, it has been reported that $PGE_2$ levels are elevated after pyrogen and anisomycin administration, even though the febrile responses were significantly attenuated (Milton and Sawhney, 1982). However, how the synthesis of new protein could account for the rapid (less than 1 minute) rise in body temperature observed after central injection of PGE is unclear, unless it is postulated that inhibition of protein synthesis affects receptor sites in the target.

Despite evidence implicating a protein mediator in the genesis of fever, it is still not clear precisely how the synthesis of new

protein contributes to the development of febrile rises in body temperature. Currently, much of the work is contradictory. Thus, further work will be required to define the role of hypothalamic protein synthesis in fever.

ii) Role of prostaglandins and the mode of action of antipyretics

The initial report concerning the pyrogenic effect of centrally administered PGE was provided by Milton and Wendlandt (1970; 1971), an action which was attributed later to an effect within the PO/AH (Feldberg and Saxena, 1971). It was proposed that PGE's might be important mediators of fever since they evoked such prompt and potent hyperthermic responses when injected into the brain. This concept was strengthened by Vane who demonstrated that non-steroidal anti-inflammatory drugs (NSAID) inhibited prostaglandin synthesis, thereby providing a mechanism to explain the fever reducing properties of commonly used antipyretics (Vane, 1971). Thus, the NSAID, acetaminophen, suppressed endotoxin fever but did not affect the hyperthermic response to centrally administered $PGE_1$.

Although the evidence implicating prostaglandins as mediators of fever is circumstantial at times, there is a large amount of experimental data supporting a role for these arachidonic acid metabolites in the genesis of fever. When injected into the PO/AH of a number of species including cats, rabbits, rats, mice, guinea-pigs, chickens, monkeys, but not the common echidna or sheep, (see Milton, 1982 for review), PGE evoked a dose related hyperthermia. In contrast, injections into the posterior hypothalamus or the midbrain reticular formation did not evoke any rise in core temperature. In an extensive exploration of sites mediating PGE hyperthermia in the rat,

Williams et al. (1977) concluded that all the active sites were located within the PO/AH, with the greatest sensitivity being found in the ventral aspects, between the anterior commissure and optic chiasm. Thus, the sites mediating the hyperthermic effects of PGE corresponded closely to those mediating the febrile effects of endogenous pyrogen (IL-1) (Cooper et al., 1967; Jackson, 1967), thereby reinforcing the idea that the PO/AH was the site where all pyrogens acted to produce fever.

However, evidence recently has been obtained suggesting that brain areas other than the PO/AH may be responsive to pyrogens, in particular prostaglandins of the E series. Stitt (1986) observed that the dose-response relationship between icv and intra PO/AH PGE was remarkably similar over a number of species when it would have been expected that injections of PGE into the PO/AH would evoke far greater rises in body temperature since this area was perceived to be the central locus of action. This, coupled with the fact that unilateral injection of PGE into the PO/AH produced rises in core temperature similar to bilateral injections (Stitt, 1973), have cast doubt on the hypothesis that the PO/AH is the most important central locus for PGE action. Instead, it has been proposed that the synthesis and interaction of PGE within the OVLT is important for fever generation. In support of this, the OVLT is very sensitive to the hyperthermic effect of exogenously administered PGE (Stitt, 1986).

The hypothesis that PGE is involved as a mediator of fever requires, firstly, that the substance be released within the CNS following the administration of pyrogens and, secondly, that this release be prevented by antipyretic drugs which can inhibit

prostaglandin synthesis. In this regard, increased concentrations of PGE in the CSF of cats (Feldberg and Gupta, 1973) and rabbits (Phillip-Dormstrom and Siegert, 1974) have been demonstrated following injection of pyrogen, an elevation which can be abolished by drugs, like aspirin (Feldberg et al., 1973), which inhibit prostaglandin synthesis (Vane, 1971). The causal relationship between fever, CSF levels of PGE and antipyretics was challenged by Cranston et al. (1975) who showed that sodium salicylate could prevent the appearance of PGE-like activity in CSF at concentrations that did not suppress fever. However, these early experiments were criticized as the withdrawal and measurement of PGE in CSF collected from the cisterna magna (a site distant from where PGE acts) was considered weak evidence for an obligatory role for prostaglandins of the E series as mediators of fever. Further, it has been claimed that the method employed to assay PGE in these earlier studies was inaccurate (Coceani et al., 1983). These latter points have been addressed since it has been demonstrated more recently that the PGE content (measured by radioimmunoassay) of third ventricular CSF increased during fever (Bernheim et al., 1980; Coceani et al., 1983), an alteration which was specific to fever and not to other thermoregulatory challenges (Bernheim et al., 1980). Since it is very difficult to measure PGE release from brain tissue in vivo, at present it is possible only to correlate CSF levels of PGE with febrile changes in body temperature. A problem with this approach is that the origin of the prostaglandins is uncertain. Prostaglandins measured in CSF may originate from areas other than the PO/AH, such as the cortex and posterior hypothalamus where microinjection of PGE does not alter core temperature.

Despite the large amount of data implicating prostaglandins of the E series as mediators of fever, there also is evidence against such a hypothesis (Mitchell et al., 1986; Cooper, 1987 for reviews). The most damaging evidence involves the prostaglandin antagonists SC 19220 and HR 546. Intracerebroventricular injections of these drugs are effective in blocking the hyperthermic effects of PGE but are unable to prevent the fevers produced by intracerebroventricular injection of either crude IL-1 (Cranston et al., 1976) or sodium arachidonate (Laburn et al., 1977), suggesting that some neurochemical other than PGE may mediate fever. These data have been criticized because of the high doses used and the route of pyrogen administration, consequently, further work will be required to clarify the significance of these results. A similar inconsistency has been reported by Veale and Cooper (1975) who observed that after bilateral lesioning of the PO/AH of the rabbit, PGE hyperthermia was abolished but crude IL-1 administered icv or iv still produced a fever of normal height, albeit of slower onset and more prolonged when compared to intact animals. In contrast, PO/AH lesions in squirrel monkeys did not alter the febrile response to icv bacterial pyrogen or PGE (Lipton and Trzcinka, 1976). A further observation regarding inconsistent actions of PGE and other pyrogens is their effects on thermosensitive neurons located with the PO/AH. This point, which has been mentioned earlier, does not support a role for PGE in the generation of fever (Eisenman, 1982 for review).

Other evidence against a role for PGE in fever is the lack of correlation in some species between the febrile effects of intracerebral PGE and intravenous pyrogens. Consequently, sheep that

responded with fever to systemic endogenous pyrogen did not always respond to intra-PO/AH PGE with a rise in core temperature (Pittman et al., 1977), even though icv PGE evoked a moderate elevation in body temperature (Bligh and Milton, 1973; Hales et al., 1973). Although these data suggest that PGE may not mediate pyrogen fevers, an alternative explanation may be that PGE does not act within the PO/AH to elevate body temperature, but rather at some other site accessible to icv infusions, e.g. the OVLT. Other species exhibiting a lack of correlation between pyrogens and $PGE_1$ include the chicken (Artunkel et al., 1977) and echidna (Baird et al., 1974).

Earlier on, the circumstantial nature of evidence implicating prostaglandins as mediators of fever was mentioned. An example is the circular argument relating inhibition of prostaglandin synthesis to antipyretics (Mitchell et al., 1986 for review). It is clear that many antipyretic drugs can inhibit prostaglandin synthesis. However, the other actions of these drugs have not been investigated thoroughly and the possibility that these other actions might account for their antipyretic properties has seldom been entertained. An exception to this was Clark and Cumby (1976) who concluded that neither acetaminophen or indomethacin exerted their antipyretic effects by inhibiting prostaglandin synthesis. Furthermore, indomethacin inhibits cyclic AMP dependent protein kinase at concentrations well below those required to inhibit cyclooxygenase (Cantor and Hampton, 1978) and floctaferine and indomethacin inhibit prostaglandin synthesis equally yet the former has far lower antipyretic action when injected icv (Laburn et al., 1980). Therefore, until it is shown conclusively that antipyretics act by inhibiting prostaglandin

synthesis (and not by some other process), it should not be assumed that the antipyretic action of NSAID provides proof that prostaglandins are the neural meditors of fever.

Evidence exists both for and against the involvement of PGE as the neural mediator of pyrogen fever. It would appear that some component of fever may rely on the synthesis and interaction of PGE with neural elements in the CNS. However, the presence of conflicting evidence indicates that this substance may not be the sole and definitive mediator of all febrile episodes. It has been suggested that other metabolites of arachidonic acid may be important for the generation of fever but up until now no such eicosanoid has been discovered (Dascombe 1985 for review). Therefore, at present, it appears that PGE is the most suitable candidate that may mediate pyrogen fever if a sole mediator is responsible.

iii) Others

Many putative neurotransmitters including noradrenaline, 5-hydroxytryptamine, acetylcholine, amino acids, peptides and cyclic nucleotides have been implicated in the central mechanisms responsible for the control of body temperature under normal and febrile conditions. It is not the aim of this section to outline all the evidence for such interactions but to discuss briefly the importance of central neurotransmitters in fever generation. An extensive review of this topic has recently been published (Dascombe, 1985).

Most of the information implicating noradrenaline and 5-hydroxy-tryptamine in fever is confusing and conflicting and at present it is not possible to make firm conclusions regarding the roles of either of these amines during fever. Conversely, the role of acetylcholine in

fever generation is less conflicting and the available evidence implicates this neurotransmitter in effector pathways involved in fever. This is because icv atropine can attenuate fever caused by systemic EP or intra PO/AH PGE in the rabbit (Cooper et al., 1976) and iv typhoid vaccine in the sheep (Bligh et al., 1977).

Although certain neuropeptides including arginine-vasopressin (AVP) and α-melanotropin (α-MSH) may modulate fever (see later), there is little evidence to support the hypothesis that any peptide (apart from IL-1) is involved in raising body temperature to febrile levels. Pyrogen fever is not reduced by the opioid antagonist naloxone (Kandasamy and Williams, 1983c) and enkephalin-like activity in the CSF of febrile cats is unaltered during pyrogen fever (Dascombe, 1985). However, levels of β-endorphin are raised in the CSF and plasma of endotoxin-treated sheep (Carr et al., 1982). Kinins (Dascombe, 1983) and other hyperthermic peptides, including somatostatin (Brown et al., 1981, thyrotrophin releasing hormone (Horita and Carino, 1975) and cholecystokinin (Kandasamy and Williams, 1983b), are unlikely to mediate fever.

Cyclic nucleotides have been investigated extensively since they function as second messengers within the CNS. However, despite much work, there is little evidence to support their involvement in fever (Dascombe, 1985 - for review).

## B. ANTIPYRETICS

Antipyretics tend to reduce febrile body temperatures towards prefebrile levels at concentrations that do not alter resting body temperature, a feature first alluded to by Ott. In addition to classical antipyretic drugs like aspirin, several endogenous neuropeptides, including AVP (Kasting et al., 1979a) and α-MSH (Glyn and Lipton, 1981), possess antipyretic activity against pyrogen fevers. The following sections will discuss evidence relating to the central actions of antipyretics.

### 1) Classical Antipyretics

#### a) Background

Antipyretic drugs may be steroidal or non-steroidal in nature, most of the commonly used antipyretics are non-steroidal, for example, aspirin, acetaminophen, antipyrine, indomethacin. The earliest antipyretic used was quinine, an extract from chinchona bark, which was used to treat malaria. The bark of another tree, the willow (Salix alba), was found to have antipyretic properties by Edward Stone. The publication of his scientific account in 1763 was the first on the use of antipyretic drugs (see Milton, 1982). The isolation of salicin from the willow bark and subsequent synthesis of salicylic acid and acetylsalicylic acid resulted in the clinical use of these antipyretic drugs towards the end of the 19th Century.

Whether fever is of benefit to the host during infection has not been determined. However, the use of antipyretic treatment has been

carried out for many years. This may be because some antipyretics, like salicylates, are not only antipyretic but also are anti-inflammatory and analgesic, thus, the pain associated with many infections may be reduced during antipyretic therapy.

b) Mechanism of Action

Antipyretics may conceivably interfere with the development of fever at any point along the febrile pathway. However, classical antipyretics do not interfere with the production or release of IL-1 (Cranston et al., 1970; Van Miert et al., 1971; Lin and Chai, 1972) nor do they inactivate the molecule (Grundman, 1969; Hoo et al., 1972; Lin and Lai, 1972) or prevent it from interacting with the CNS (Cranston et al., 1970; Lin and Chai, 1972; Clark and Cumby, 1975). Since antipyretics are effective when injected into the PO/AH in much smaller doses than required iv (Cranston et al., 1970; Lin and Chai, 1972), and since salicylate injected into the PO/AH inhibited the effects of crude IL-1 injected into the same area (Schoener and Wang, 1975), it was proposed that salicylate acted within this area of the brain. An action of antipyretics within the PO/AH is supported further since acetylsalicylate can reverse pyrogen induced changes in neuronal activity in this area (see earlier). (Wit and Wang, 1968; Cabanac et al., 1968; Eisenman, 1969).

If prostaglandins mediate pyrogen fever (see earlier), then evidence exists that antipyretics act by inhibiting prostaglandin synthesis (Vane, 1971). In support of this, there is a significant correlation between antipyretic activity and inhibition of prostaglandin synthesis (Ziel and Krupp, 1975) and antipyretics do not

alter the hyperthermic effect of prostaglandins once synthesized (Milton, 1982). However, the latter point may no longer be valid since it has been demonstrated recently that intraseptal (VSA) infusions of salicylate block PGE-induced hyperthermia (Alexander et al., 1987).

2) Endogenous Antipyresis and the Role of Vasopressin

It has long been recognized that fevers rarely exceed 4°C above normal body temperature (Du Bois, 1949). This suggests that the body may possess some form of negative feedback which controls the magnitude of the febrile response. Indeed, an excessive rise in body temperature during fever may result in permanent CNS damage or, in children, febrile convulsion. Certain endogenous substances, including cortisone (Atkins et al., 1955) and cortisol (Chowers et al., 1968) have been shown to be antipyretic. However, the next section will discuss the concept of endogenous antipyresis (Kasting et al., 1979c) involving the neurohypophyseal peptide arginine vasopressin.

a) Fever at Term and in Neonates

Numerous reports have indicated that newborn humans may not develop fever in response to severe infection, including gastroenteritis (Epstein et al., 1951) or septicemia (Smith et al., 1956). This lack of fever has often been attributed to an immaturity of the thermoregulatory system. However, the lamb and guinea-pig, two species with a relatively mature thermoregulatory system at birth

(Alexander and Williams, 1968; Blatteis, 1975), generate reduced fevers when compared to adults of the same species (Pittman et al., 1974; Blatteis, 1975). This diminished febrile response is evident following either systemic or central (Blatteis and Smith, 1979) administration of pyrogens. In addition, the febrile response to either endotoxin or endogenous pyrogen (IL-1) is reduced in the pregnant Suffold cross ewe (Kasting et al., 1978) and guinea-pig (Zeisberger et al., 1981) from about 4-5 days prepartum to about 5 hours after birth. Since white blood cells of either adults or neonates retain their ability to produce endogenous pyrogen (Blatteis, 1977; Kasting et al., 1979b; Dinarello et al., 1981) during this periparturient period, it was postulated that the decreased febrile responses at term were due to increased levels of an endogenous antipyretic substance. Of the many hormonal fluctuations occurring during pregnancy, that of vasopressin corresponded most closely to the periods where reduced fevers were observed (Alexander et al., 1974) and was therefore investigated subsequently for antipyretic activity in the brain (Kasting et al., 1979a). In addition, anatomical evidence demonstrated that activation of central vasopressinergic pathways occurred in the pregnant guinea-pig at term. Specifically, levels of AVP (determined immunocytochemically) increased in neurons of the paraventricular nucleus and within nerve terminals of the amygdala and septum (Merker et al., 1980), increases which may be responsible for the antipyretic response observed at term.

The other endogenous substances known to be antipyretic, e.g. cortisol, are unlikely to be involved in the antipyresis observed at term since the plasma levels of this steroid do not increase with a

time course similar to that observed for the decreased fevers in the ewes (Jones et al., 1977; Liggins et al., 1973).

b) Evidence Supporting a Role for Vasopressin in Endogenous Antipyresis

In the following sections, evidence supporting a role for vasopressin in fever suppression in non-pregnant animals will be outlined. The evidence will be divided into three groups: 1) locus of action, 2) physiological, and 3) pharmacological. In addition, possible mechanisms of the peptide-induced antipyresis will be discussed.

i) Locus of action

A functional role for vasopressin in fever suppression in the non-pregnant adult has been demonstrated in the sheep. When vasopressin is perfused within the VSA of the sheep brain, the fever evoked by systemic bacterial pyrogen is suppressed in a concentration dependent fashion (Kasting et al., 1979a). The brain site where exogenously administered vasopressin suppresses fever is located similarly over a number of species (see later), namely lateral to the vertical limb of the diagonal band of Broca and immediately ventral to the lateral septum. The antipyretic action of vasopressin is observed only when the peptide is perfused within this discrete region of the brain. When AVP is administered outside the VSA, into the lateral, anterior or posterior hypothalamus, the preoptic area, the fornix (Cooper et al., 1979), or the dorsal septum (Bernardini et al., 1983), the normal development of the febrile response is unaffected. The antipyretic locus of action is very discrete, indeed, failure to

observe an antipyretic action of AVP when injected into the dorsal septum of the rabbit was perceived by Bernardini et al. (1983) to suggest that the fever reducing properties of AVP were species specific. Evidence that the antipyretic action of vasopressin is centrally mediated is that intravenous administration of the peptide does not result in any suppression of fever (Cooper et al., 1979).

Initial observations by Cushing (1931a) demonstrated that injection of "pituitrin" into the cerebral ventricles of humans induced a decrease in body temperature of febrile patients which was coincident with marked sweating and vasodilation. Interestingly, Cushing described a patient suffering from a glial tumor which had destroyed completely the septum and the anterior part of the third ventricle (Cushing, 1931b). Under these circumstances, injection of pituitrin did not produce any effect on rectal temperature or any other autonomic function. Cushing (1931b) explained these observations as follows: "This, then was a case in which pituitrin and pilocarpine introduced into the ventricle failed to have their usual stimulatory effect, presumably owing to the neoplastic destruction of the centers concerned in the normal reaction". This may be the first description of a septal involvement in vasopressin-induced antipyresis.

Other studies employing the ventricular route of administration also have described an antipyretic action for centrally administered vasopressin (Kovacs and De Wied, 1983; Kandasamy and Williams, 1983a; Kasting and Wilkinson, 1986). In the rat, this antipyretic effect is observed at concentrations well below those required to induce hypothermia (Kruk and Brittain, 1972; Kasting et al., 1980; Meisenberg

et al., 1984a), suggesting that the antipyretic effect of vasopressin is unlikely to be due simply to a non-specific fall in core temperature. However, in contrast to the rat, when vasopressin is injected similarly into the rabbit (Bernardini et al., 1983) or monkey (Lee et al., 1985), no consistent reduction in the febrile response is observed. This absence of antipyretic action following intracerebroventricular injection of AVP may reflect an inability of the peptide to reach important antipyretic sites within the VSA in these species. Thus, the full spectrum of thermoregulatory actions of vasopressin (and other peptides) may not be revealed after intracerebroventricular administration. In support of this, intrahypothalamic vasopressin evokes hyperthermia (Lin et al., 1983), a response which is at odds to the antipyretic action of intraseptal AVP unless strict attention is made to their neuroanatomical loci of action, i.e. central vasopressin exerts a very different action dependent upon where it is injected.

ii) Physiological evidence

The release of endogenous vasopressin from AVP sensitive antipyretic sites in the sheep brain during fever has been used as evidence that AVP functions under physiological conditions as an endogenous antipyretic. During fever, the amount of AVP in returned push-pull perfusates obtained from the VSA correlated negatively with changes in body temperature (Cooper et al., 1979). Therefore, as body temperature rose, less AVP was present in the extracellular fluid. Similarly, as body temperature fell, the amount of AVP released into the septal perfusates increased. A comparable negative correlation between AVP release and febrile response has been reported for the

rabbit (Ruwe et al., 1985). The levels of AVP in CSF also show changes during fever. In this case, however, the amount of AVP present in the CSF correlated significantly with increases in body temperature (Kasting et al., 1983). Cerebrospinal fluid sampling may, because of the collection from a larger area and because of the possible involvement of one neurotransmitter in a number of neuronal circuits, reveal a profile of release which is different from that at one specific site of release. However, that changes in vasopressin levels occur during fever in the brain area where exogenous AVP reduces fever are strong indicators of a physiological role for this peptide in the central mechanisms controlling core temperature.

Immunocytochemical investigation of AVP neuronal systems in the brain also demonstrates changes during fever . Following injection (im) of Salmonella enteritidis pyrogen into guinea-pigs, an activation of ascending projections to the septal area and the amygdala was observed (Zeisberger et al., 1986), changes which are similar to those observed when fever is absent at term (Merker et al., 1980). This activation of vasopressinergic neurons in response to pyrogen preceded peak fever. However, it is difficult to correlate the presence of vasopressin in terminals and axons with the time-course of the febrile response since actual release of peptide is not measured. However, that AVP projections to the septal area are activated during fever strongly supports the hypothesis that this peptide may be involved as a neurotransmitter in this area of the brain during fever.

Changes in tissue vasopressin levels in various brain areas during fever also have been demonstrated. In response to an iv injection of bacterial endotoxin, the concentration of vasopressin

decreased in the septum, amygdala and caudate nucleus of the rat, while the concentration of AVP-like material remained unaltered in other areas (Kasting and Martin, 1983), supporting the hypothesis that vasopressin may function as a neurotransmitter during fever.

In addition to measuring the endogenous release of vasopressin during fever, antagonist analogues can be utilized to prevent the normal actions of endogenously released AVP. During fever, perfusion of a specific AVP-antiserum (Kasting, 1980; Malkinson et al., 1987) or a weak AVP antagonist (desamino dicarba AVP) (Kasting, 1980) in AVP sensitive antipyretic sites in the VSA enhanced the febrile response, presumably by preventing endogenously released vasopressin from modulating fever by interfering with its interaction with receptors in the ventral septum. Thus, these data indicate that sequestering endogenously released vasopressin with a specific antibody or blocking AVP receptors in the VSA effectively produces the same end result. Similarly, augmenting vasopressin release by haemorrhage (Kasting et al., 1981) or peripheral hypertonic (Kasting, 1986) saline results in a reduction in the magnitude of fever.

Electrical stimulation of one potential source of AVP in the VSA, the paraventricular nucleus (Disturnal et al., 1985), blocked the rise in temperature normally associated with systemic injection of bacterial pyrogen (Ruwe et al., 1986). Thus, stimulation of the paraventricular nucleus mimicked the antipyretic effect of exogenously administered vasopressin. However, before firm conclusions are made regarding any potential connection between these two events, it will be necessary to verify that the stimulus-induced antipyresis was due to increased release of vasopressin within the VSA. This could be

achieved with either specific vasopressin antagonists or push-pull cannulae and a suitable assay.

In addition to providing evidence for putative sources of vasopressin in the VSA (Disturnal et al., 1985), single unit recording techniques have demonstrated that thermoresponsive neurons exist within the ventral septum (Disturnal et al., 1986) whose activity can be modified by stimulation of putative AVP-projections from the paraventricular nucleus and bed nucleus of the stria terminalis (Disturnal, 1986). Further electrophysiological evidence that vasopressin might be associated with thermoregulation in the septum is the recent finding that iontophoretic application of AVP onto neurons within the ventral septum can inhibit the excitatory effects of glutamate within the VSA, an action blocked by a $V_1$ antagonist (Disturnal et al., 1987). Therefore, that some AVP responsive cells are inhibited by inputs from the paraventricular nucleus, the bed nucleus of the stria terminalis and iontophoretically applied exogenous AVP, suggest that vasopressin may be a transmitter in this pathway. However, this requires further experimentation using AVP antagonists before firm conclusions can be made.

iii) Pharmacological evidence

The antipyretic action of vasopressin is specific to the hormonally active peptide. Neither a behaviourally active fragment, DGAVP (des-9-glycinamide-arginine-vasopressin), nor the structurally similar peptide oxytocin (Kovacs and De Wied, 1983) exhibited antipyretic activity against pyrogen fevers. It likely involves a receptor mediated process as the antipyresis is dose-related (Cooper et al., 1979; Kovacs and De Wied, 1983) and can be blocked by a

vasopressor (Kruszynski et al., 1980) AVP antagonist (Kasting and Wilkinson, 1986). Further evidence supporting a specific action of vasopressin is that other endogenous neuropeptides including somatostatin, substance P and angiotension II are ineffective in reducing fever (Kasting, 1980). However, a more thorough investigation will require the use of agonists and antagonists of vasopressin.

c) Mechanisms of Vasopressin Induced Antipyresis

The precise mechanisms involved in the antipyretic actions of AVP have not been demonstrated conclusively since the data are incomplete and often conflicting. However, vasopressin might influence core temperature during febrile conditions by 1) altering the set point for body temperature control, 2) decreasing heat production and, 3) increasing heat loss, or a combination of all three.

In the rat, evidence exists to suggest that the antipyretic effect of intracerebroventricularly administered AVP (like indomethacin) is primarily a result of a change in the febrile set point coupled with an inhibition of heat production (Wilkinson and Kasting, 1986). Alternatively, based on the effects of preoptic cooling and exposure to different ambient temperatures, Banet and Wieland (1985) concluded that application of AVP into the lateral septum acted to inhibit thermoregulatory heat production rather than to alter normal set point temperature. The different conclusions of these two studies may be a result of the different modes of vasopressin administration; intracerebroventricular versus intraseptal. Furthermore, the septal injections described by Banet

and Wieland (1985) were located in regions of the dorsal septum not responsive to the antipyretic effect of AVP (see later) and the dose used was some 200-2000 times more than that required for antipyresis (Kovacs and De Wied, 1983; Wilkinson and Kasting, 1986; see later).

Vasopressin has not been reported to increase heat loss during fever and so it is unlikely that this represents a means whereby this peptide exert its antipyretic effect. However, before firm conclusions regarding the precise mechanisms of vasopressin induced antipyresis are formulated, further investigations involving the injection of AVP into antipyretic-active sites in the septum over a number of ambient temperatures will be required.

d) Convulsive Disorders and Vasopressin

Injection of vasopressin into the lateral cerebral ventricles of rats evokes, via a sensitization process, convulsive behaviour characterized by myoclonic/myotonic seizures and barrel rotations (Kruse et al., 1977; Kasting et al., 1980). These convulsive episodes are coincident with an immediate increase in the amplitude and a decrease in the frequency of hippocampal electrical activity followed by cortical spiking (Kasting, 1980; Burnard et al., 1983).

The specific neuroanatomical locus mediating this action of AVP is found within the VSA (Naylor et al., 1985), the area of the brain where AVP exerts its antipyretic effects. The fact that AVP is released centrally during fever (Cooper et al., 1979; Kasting et al., 1983), along with its convulsive actions in the same area of the brain, raised the possibility that vasopressin might be involved in the etiology of febrile convulsions (Kasting et al., 1981). In

support of this, a lack of AVP, as found in Brattleboro rats (Valtin et al., 1965), increased the threshold for hyperthermic convulsions (Kasting et al., 1981) and a vasopressin antagonist that blocked the convulsive effects of AVP (Naylor et al., 1985; Burnard et al., 1986) also blocked the antipyretic effect of vasopressin (Kasting and Wilkinson, 1986). Although the evidence implicating vasopressin as a mediator of febrile convulsions is largely circumstantial, it is possible that the AVP released as an endogenous antipyretic during fever may contribute to febrile convulsions.

3) Evidence Supporting a Role for α-MSH in Fever Suppression

α-melanotropin, a peptide derived from pro-opiomelanocortin, may play a role in central temperature control mechanisms concerned with fever since α-MSH can reduce pyrogen fever in the rabbit (Glyn and Lipton, 1981), guinea-pig (Kandasamy and Williams, 1984), but not the cat (Rezvani, et al., 1986).

During maximal fever, there is an elevated level of α-MSH within the dorsal septum (Samson et al., 1981), but not the anterior hypothalamic preoptic area, which is specific to febrile rises in body temperature and not to hyperthermia (Holdeman et al., 1985). Furthermore, microinjection of exogenous α-MSH into this septal region can limit fever in the rabbit (Glyn-Ballinger et al., 1983). Studies on the release of α-MSH from septal sites during fever have not been carried out yet but intracerebroventricular injection of α-MSH antiserum, for 3 days prior to interleukin-1 injection, resulted in markedly prolonged fevers, presumably by preventing the antipyretic

action of endogenously released α-MSH (Shih et al., 1986). How an action of α-MSH might modulate fever is not known but may be via neuroanatomical connections with the primary temperature control centres in the hypothalamus (Eskay et al., 1979) involving an alteration of thermoregulatory set-point (Richards and Lipton, 1984). However, α-MSH does not attenuate the hyperthermia evoked by one putative mediator of fever, prostaglandins of the E series (Clark et al., 1985).

The development of antagonists to α-MSH and also information on the temporal release of the peptide during fever will enable a thorough investigation and the formulation of more conclusions on the role of α-MSH in the central mechanisms controlling core temperature.

## C. VASOPRESSIN AS A NEUROTRANSMITTER IN THE VENTRAL SEPTAL AREA

Postulation of a transmitter role for vasopressin in the VSA requires that the criteria for a neurotransmitter candidate be fulfilled for AVP in this area of the brain. In the previous section, physiological and pharmacological evidence supporting a role for vasopressin as an endogenous antipyresic in the VSA was outlined. This section will outline the evidence supporting the hypothesis that AVP acts as a neurotransmitter within the VSA.

1) Anatomy and Sources of Vasopressin in the Ventral Septum

The major sources of extrahypothalamic vasopressin in the brain are thought to derive from the paraventricular nucleus (PVN), suprachiasmatic nucleus (SCN), and bed nucleus of the stria terminalis (BST) (Swanson, 1977; Buijs et al., 1978; Sofroniew and Wiendl, 1978; Van Leeuwen and Caffe, 1983; De Vries and Buijs, 1983; De Vries et al., 1985), although cell bodies outside these areas also have been described. In addition to immunocytochemistry, lesion and electrophysiological studies have been used to investigate the central projections and terminations of potential AVP-containing neurons. Although electrophysiological evidence exists for PVN-septal projections (Pittman et al., 1981; Disturnal et al., 1985), lesion studies do not support the vasopressinergic nature of the pathway in the rat (De Vries and Buijs, 1983). Current immunocytochemical evidence implicates the BST as a major source of AVP to the lateral and ventral septum (De Vries et al., 1985). This is supported by lesion (De Vries and Buijs, 1983), electrophysiological (Disturnal et al., 1985) and retrograde transport studies (De Vries and Buijs, 1983).

Therefore, at present, it appears that the vasopressin located in the VSA is derived primarily from the BST. In support of a neurotransmitter role for AVP in the ventral septum, this area of the brain is composed largely of AVP-containing terminals, rather than fibres or cell bodies (De Vries et al., 1985).

2) Synthesis

Vasopressin, like most peptide hormones, is derived from a precursor molecule which consists of a pre-pro-hormone synthesized on the ribosomes. This precursor molecule is composed of a signal peptide, an AVP sequence, a neurophysin sequence and a glycoprotein portion (Land et al., 1982; Schmale et al., 1983). Further processing to yield smaller peptides occurs during axonal transport to the terminals by various processing enzymes. This is what occurs in the hypothalamo-neurohypophyseal system. It is unclear as to whether the same process occurs in extrahypothalamic projections since extensive biochemical characterization of AVP synthesis under these conditions has not been carried out. However, the available evidence suggests that extrahypothalamic vasopressin may be synthesized in a similar way to the hypothalamus (Pickering et al., 1983), although the processing may be different (Sinding et al., 1982).

3) Release Upon Stimulation

Vasopressin has been identified, by immunocytochemistry and electron microscopy, in terminals which make synaptic connections with dendrites of neurons in various areas of the brain, including the septum (Buijs and Swaab, 1979). To fulfill the criteria for a neurotransmitter, stimulation of afferent input or depolarization should cause the release of the neurotransmitter from nerve terminals. In this regard, either veratridine or high $K^+$ stimulated AVP release *in vitro* from the septum via a $Ca^{++}$ dependent process (Buijs and Van

Heerikhuize, 1982). In addition, electrical stimulation of the PVN or supraoptic nucleus evokes AVP release in the spinal cord (Pittman et al., 1984) and lateral septum (Demotes-Mainard et al., 1986), respectively, the latter by a calcium dependent process. Experiments where either the PVN or BST were stimulated and AVP release measured in the VSA have not been undertaken. However, the release of vasopressin from the VSA under physiological conditions has been demonstrated during fever (Cooper et al., 1979; Kasting and Martin, 1983; see earlier), observations which are consistent with the hypothesis that vasopressin might act within this area of the brain as a neurotransmitter. Release of AVP from the septum also occurs in response to hemorrhage or peripheral hypertonic saline, two potent stimuli for the peripheral secretion of AVP (Pittman et al., 1982; Demotes-Mainard et al., 1986).

4) Receptors for Vasopressin

The many documented behavioural and physiological actions of intracerebral vasopressin have provided indirect evidence for the presence of central AVP receptors. However, direct evidence from autoradiographic and radioligand binding studies exists for vasopressin receptors in the brain (Yamamura et al., 1983; Dorsa et al., 1983; Pearlmutter et al., 1983; Biegon et al., 1984), including the ventral septum where vasopressin is antipyretic (Baskin et al., 1983). Binding is reversible, saturable, of high affinity and specific to vasopressin (Audigier and Barberis, 1985). The effects of AVP also show structure activity relationships (Abood et al., 1980; De

Wied et al., 1984; Meisenberg and Simmons, 1984a), a feature which strengthens the hypothesis that vasopressin exerts its effects by interacting with receptors.

In the periphery, two subtypes of vasopressin receptor have been well characterized ($V_1$-vasopressor, $V_2$-antidiuretic), based on differing ligand selectivities and the second messenger involved (Mitchell et al., 1979; Brown et al., 1963; Kirk et al., 1979). The nature of the receptors in the VSA has not been investigated thoroughly. However, injection of a $V_1$ receptor antagonist into this area blocks the convulsive effects of icv AVP (Naylor et al., 1985). At present, no action attributed to an effect on $V_2$ receptors has been described for this area. These latter observations, on the predominance of the $V_1$ receptor subtype in the VSA, are in agreement with displacement studies using $V_1$ and $V_2$ vasopressin analogues (Poulin and Pittman, 1986; personal communication).

5) Inactivation of Vasopressin

Although it was considered that all neurotransmitters required a mechanism of inactivation, this may not be the case for neuropeptides whose actions may often be prolonged and modulatory. However, proteolytic enzymes are found in abundance in the brain so neuropeptides could be inactivated by breakdown. Both intact AVP and fragments of AVP exert biological actions in the brain (De Wied, 1976; Kovacs and De Wied, 1983), suggesting that proteolytic processing may be important for more than just breakdown. Aminopeptidases located in the brain are considered responsible, firstly, for the generation of

such neuroactive AVP fragments and, secondly, for their ultimate breakdown (Burbach and Lebouille, 1983).

6) Actions of Vasopressin on Ventral Septal Neurons

The actions of vasopressin on ventral septal neurons have not been investigated thoroughly. Iontophoretic application of vasopressin onto neurons in the ventral septum caused a reduction in the excitatory response of these neurons to glutamate (Disturnal and Pittman, 1987), a response which was mimicked by stimulation of BST afferent projections (Disturnal and Pittman, personal communication). In the lateral septum results are less clear since both excitation (Joels and Urban, 1982; Joels and Urban, 1984) and inhibition of neuronal activity have been reported (Marchand and Hagino, 1982). Similar conflicting results have been reported for the effects of AVP on hippocampal neurons (Mühlethaler et al., 1982; Mühlethaler et al., 1983; Mizuno et al., 1984).

The actions of AVP on central neurons are not clear. Further work, especially in the VSA, will be required in order to understand fully the putative neurotransmitter role of vasopressin in this area of the brain.

D. RESEARCH OBJECTIVES

This research was undertaken to investigate further the hypothesis that vasopressin might function within the brain as an endogenous antipyretic. Therefore, experiments were designed:

1) to investigate the interspecies variability of vasopressin-induced antipyresis using the rabbit, rat, cat and guinea-pig.

2) to locate sites in the brain where vasopressin suppressed fever and to relate these to studies where vasopressin-induced antipyresis was not reported.

3) to characterize the thermoregulatory actions of vasopressin in the rat, relating specific changes in core temperature to specific brain areas

4) to determine whether vasopressin could suppress the fever evoked by one putative mediator of fever, prostaglandins of the E series.

5) to examine the dose-related nature of vasopressin-induced antipyresis and to determine the antipyretic action of the structurally related peptide, oxytocin.

6) to characterize the nature of the central receptor responsible for the antipyretic action of vasopressin using relatively specific agonists and antagonists directed against the peripheral $V_1$ and $V_2$ subtypes of vasopressin receptor.

7) to determine the role of endogenous vasopressin in fever suppression using vasopressin antagonists.

8) to investigate the mechanism of vasopressin-induced antipyresis.

## II. ANTIPYRETIC ACTION OF VASOPRESSIN IN THE RABBIT

### A. EFFECTS OF INTRASEPTAL AND INTRACEREBROVENTRICULAR VASOPRESSIN ON ENDOTOXIN FEVER

#### 1) Introduction

Although perfusion of AVP within the VSA suppresses endotoxin fever in a dose-dependent fashion in the sheep (Kasting et al., 1979a), a recent report concerning the antipyretic action of AVP in the rabbit indicates that microinjections of this neuropeptide into the ventricular system or into the dorsal septum are not antipyretic but actually enhance the febrile response to leukocyte pyrogen administered intravenously (Bernardini et al., 1983). The present experiments were carried out to determine whether AVP could suppress a pyrogen fever in the rabbit by perfusing the peptide in sites in the brain similar to those in which AVP has been shown to be antipyretic in the sheep. Specifically, the discrepant observations concerning the antipyretic effect of AVP in the rabbit were investigated to define the site where this neuropeptide might act to modulate the febrile response. In addition, the effects of intracerebroventricularly administered AVP on the development of an endotoxin fever were investigated.

2) Methods

Male New Zealand White rabbits were housed individually and maintained on a light/dark cycle of 12 hours. Food and water were available ad libitum.

Bilateral stainless steel guide cannulae (20 ga) were implanted stereotaxically above the septum of rabbits (2.5-2.9 kg), under pentobarbital anaesthesia (45-60 mg/kg), according to the stereotaxic coordinates of Sawyer et al. (1954). An additional guide cannula was implanted above a lateral cerebral ventricle and all cannulae were affixed to to the calvaria with dental cement and stainless steel screws. A polyethylene cap was also attached to the skull with stainless steel screws to protect the array of cannulae. Stylettes were inserted to occlude the cannulae when they were not in use. Rabbits were given penicillin post-operatively and were allowed at least 10 days to recover after surgery. During experimentation, the rabbits were restrained lightly with a neck restrainer (Hampton et al., 1973) to which they had become previously accustomed.

The body temperature was measured using a thermistor probe (YSI 701) inserted 10 cm beyond the anus and taped to the tail. Ear skin temperature was monitored also as a crude indicator of peripheral vasomotor tone using a YSI 709 probe taped to the ear. These temperatures were monitored throughout the experiments and recorded at 5 minute intervals on a digital datalogger (United Systems Digitec).

A push-pull cannula system (see Figure 1) was utilized for the perfusion of brain tissue. The system consisted of a 23 ga outer cannula for withdrawal and an inner 30 ga cannula for infusion, which

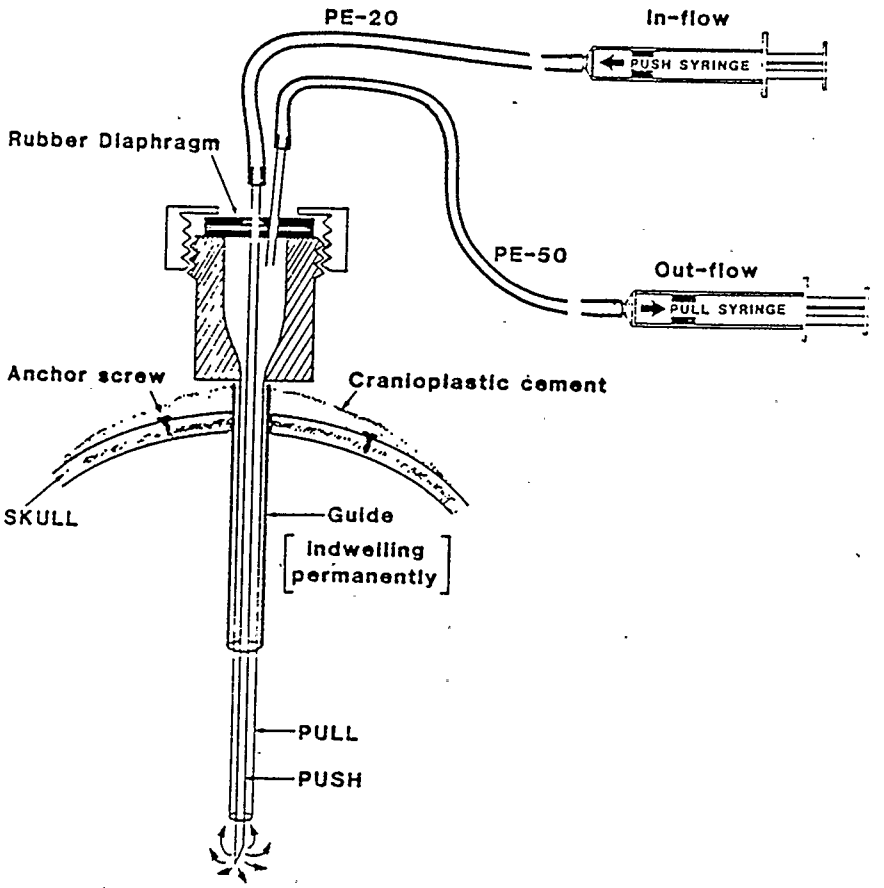



FIGURE 1 Diagram of the push-pull perfusion cannula used for larger animals such as rabbits and cats.

extended 0.5-1.0 mm beyond the outer cannula. Gas tight syringes (Hamilton) were mounted on a Harvard infusion-withdrawal pump which had been calibrated previously to deliver 30-35 µl of the perfusion medium per minute to the tissue. The solutions used for push-pull perfusion were sucrose (260 mM) and sucrose containing AVP (5.0 µg/ml). Synthetic AVP (Bachem) was stored in a stock solution of 0.025 M acetic acid (5.0 mg/ml) and was diluted as required. The bacterial pyrogen was derived from Salmonella abortus equi (SAE, Lot No. 648406; Difco Laboratories) which was diluted in sterile, pyrogen free, physiological saline.

For iv injections of bacterial pyrogen, a catheter was placed in the marginal ear vein prior to the beginning of the experiment so that the pyrogen (75 ng SAE/0.75 ml) or the control solution (sterile saline, 0.75 ml) could be delivered without disturbing the animal further. Injection of the pyrogen and the start of bilateral push-pull perfusion occurred simultaneously. The perfusion continued for 180 min following this injection. No injection or perfusion started before a control period of 120 min had elapsed, which allowed the animal to develop a stable baseline of core temperature. The order of perfusion for the vehicle and the vehicle containing AVP was randomized with a week between treatments.

For icv injections of bacterial pyrogen a needle was lowered through the ventricular cannula. Sterile saline (10 µl) containing 10 ng SAE or 10 µl of the sterile saline alone was administered by gravity flow into a lateral cerebral ventricle. One hundred and twenty minutes prior to this injection, bilateral push-pull perfusion of the ventral

septal area with either vehicle or vehicle plus AVP was started. The perfusion continued for an additional 180 min following injection of the pyrogen.

For icv injections of AVP (5.0 µg/10 µl), the peptide was dissolved in nonpyrogenic, isotonic saline and delivered by a gravity flow into the lateral cerebral ventricle in a volume of 10 µl 30 min after an iv injection of SAE (100 ng/1.0 ml). As a control, 10 µl of saline were injected similarly into a lateral ventricle 30 min after the bacterial pyrogen.

For histological verification of the perfusion sites, each animal was anaesthetized deeply and its brain perfused through the heart with 0.9% saline followed by formalin. After removal, the brain was sectioned on a freezing microtome and the sections were mounted on glass slides and stained. The site of each perfusion was localized using light microscopy.

Results were analyzed statistically using a paired Student's t-test. Fever indices were expressed as area under the fever curve in °C.h (for 5 hours).

3) Results

Figure 2 illustrates the mean core temperature responses following an iv injection of the control solution (saline) and during bilateral perfusion of the VSA with 5.0 µg/ml AVP, the concentration of AVP tested for antipyresis. This concentration of AVP had no effect on the core temperature of the afebrile rabbit when perfused in these discrete sites in the brain.

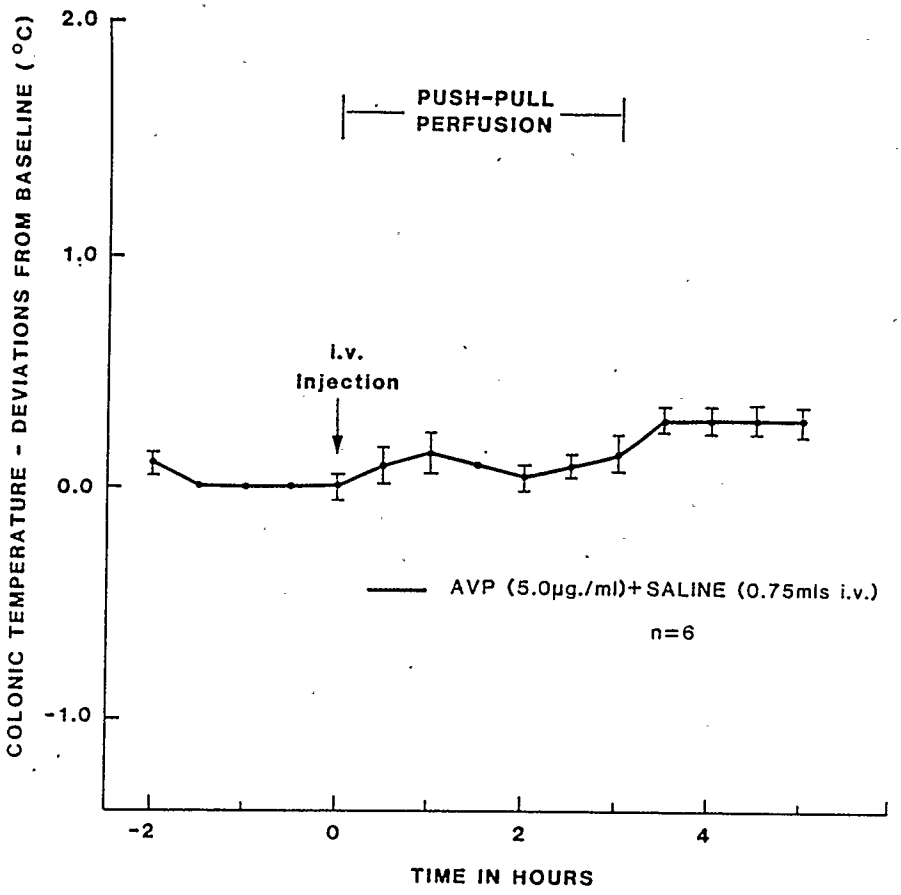



FIGURE 2 Mean colonic temperature responses (± SEM) observed in 6 rabbits. AVP at a concentration of 5.0 µg/ml was perfused into the VSA immediately prior to and for 3.0 hr after an intravenous injection of saline (0.75 ml) at time zero.

FIGURE 3 Colonic temperature in deviations from baseline (°C) in two rabbits. AVP at a concentration of 5.0 µg/ml (open circles) or the vehicle solution alone (sucrose; closed circles) was perfused into the VSA immediately prior to and for 3.0 hr after an intravenous injection of SAE (75 ng) at time zero. Perfusion of AVP after an intravenous injection of saline (open triangles) was without effect on resting body temperature. Loci of perfusions are indicated by closed circles on the histological insets. Abbreviations: AC-Anterior Commissure; CN-Caudate Nucleus; COR-Cortex; DBB-Diagonal band of Broca; IC-Internal Capsule; OC-Optic Chiasm; POA-Preoptic Area; PU-Putamen; SP-Septum Pellucidum.

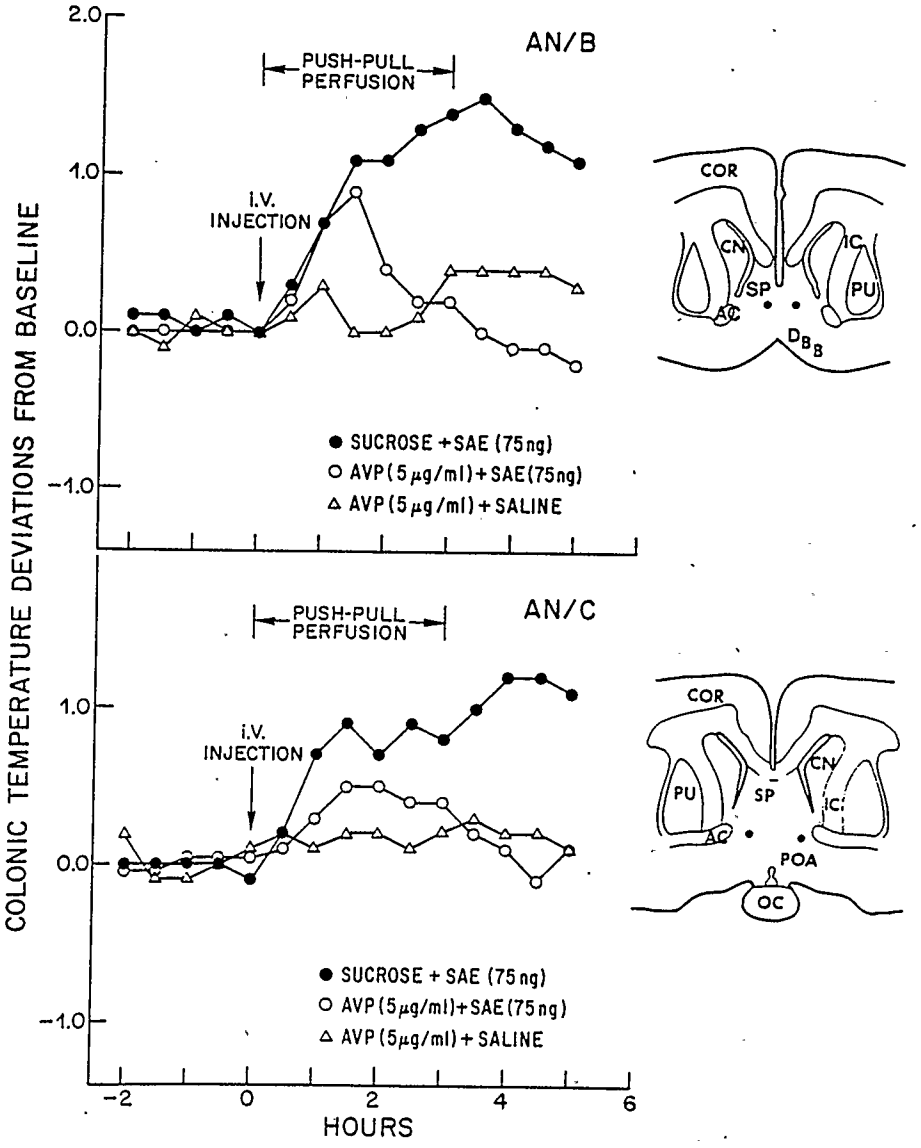

AN/B
PUSH-PULL PERFUSION
i.V. INJECTION
2.0
1.0
0.0
-1.0
● SUCROSE +SAE (75ng)
○ AVP(5μg/ml)+SAE(75ng)
△ AVP(5μg/ml)+SALINE
COR
CN
IC
SP
PU
AC
DBB
AN/C
PUSH-PULL PERFUSION
i.V. INJECTION
1.0
0.0
-1.0
● SUCROSE + SAE (75ng)
○ AVP(5μg/ml)+SAE(75ng)
△ AVP(5μg/ml)+SALINE
COR
CN
PU
SP
IC
AC
POA
OC
-2
0
2
4
6
HOURS
COLONIC TEMPERATURE DEVIATIONS FROM BASELINE

Figure 3 shows the time course of the febrile response observed in two individual rabbits injected with 75 ng SAE iv either in the presence or absence of AVP (5.0 µg/ml). Perfusion of the carrier vehicle alone did not alter the characteristic biphasic fever. However, when AVP was perfused similarly, an initial increase in core temperature occurred which was followed by a rapid return to baseline. Figure 4 shows both the colonic and ear skin temperatures during fever in the presence and absence of AVP. After an initial rise in core temperature, associated with vasoconstriction, the core temperature returned towards baseline levels when AVP was perfused in the ventral septum but not when the vehicle alone was perfused in these same sites. The antipyresis observed when AVP was present was not associated with any further alterations in the vasculature of the ear (when compared to control responses without AVP). Three characteristics of the febrile response after iv pyrogen are depicted in Figure 5, where the data obtained from 5 animals have been grouped together. Each of the three indicators of the magnitude of fever were reduced significantly after perfusion with AVP.

Experiments carried out in a separate group of animals from those previously described, demonstrate that the maximum fever height and the fever index were significantly reduced ($p < 0.05$) when AVP was perfused in the VSA in conjunction with an icv administration of SAE in each of three rabbits. A comparison of the effects of AVP, or the vehicle, on the characteristics of the febrile response are presented in Figure 6.

The schematic histological sections presented in Figure 7 illustrate the sites where perfusion of AVP suppressed a pyrogen

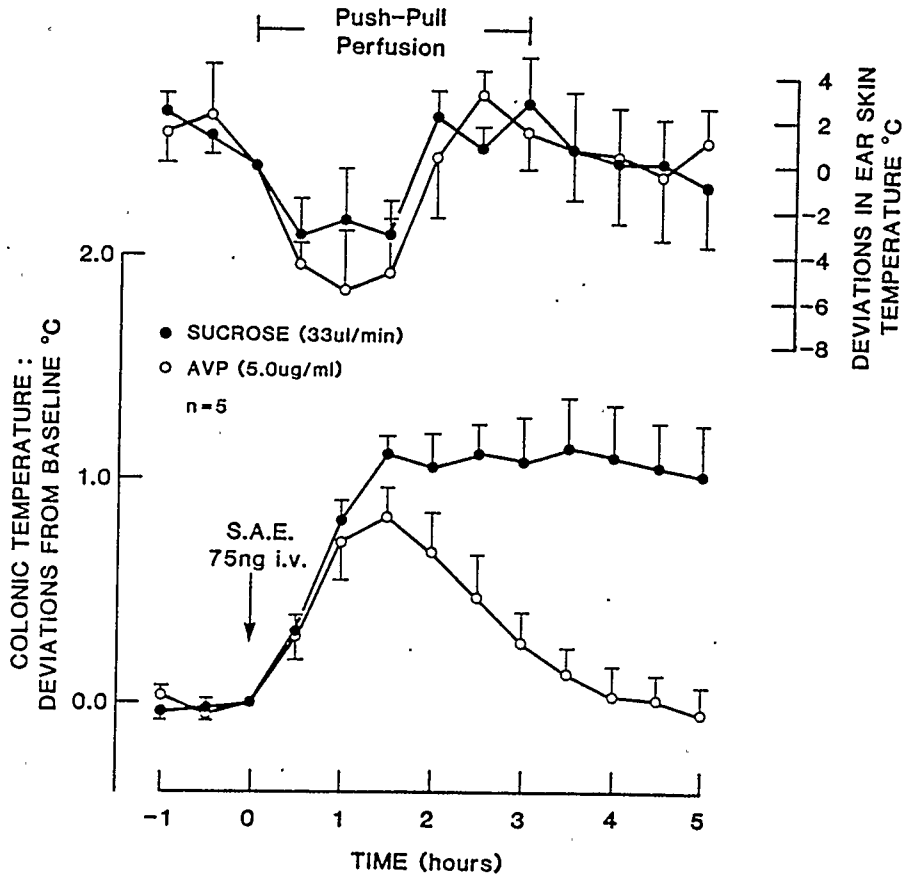



FIGURE 4 Mean colonic and earskin temperature responses (± SEM) observed in 5 rabbits. AVP at a concentration of 5.0 µg/ml (open circles) or the vehicle solution alone (sucrose; closed circles) was perfused into the VSA immediately prior to and for 3.0 hr after an intravenous injection of SAE (75 ng) at time zero.

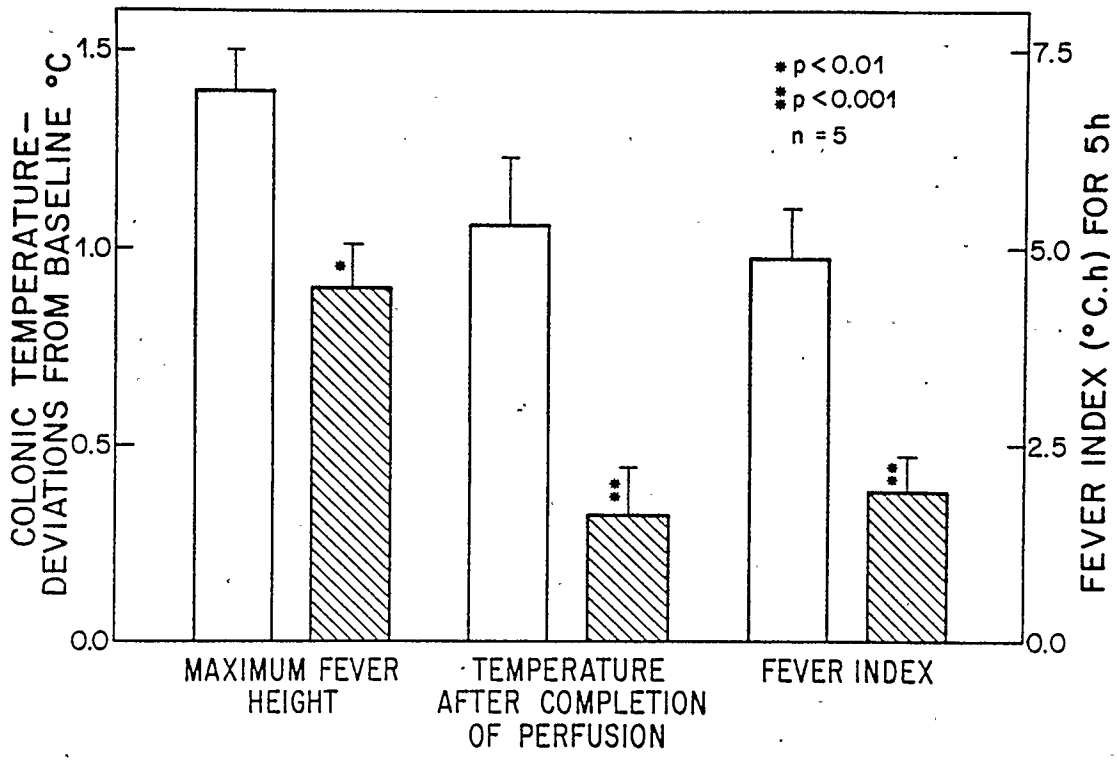



FIGURE 5 Fever responses (± SEM) of rabbits to intravenous SAE during push-pull perfusion with sucrose (open bars) and sucrose containing AVP (striped bars; n=5). Maximum fever height, the temperature after completion of perfusion and the fever index were significantly decreased when AVP was present. (*p < 0.01; ** p < 0.001).

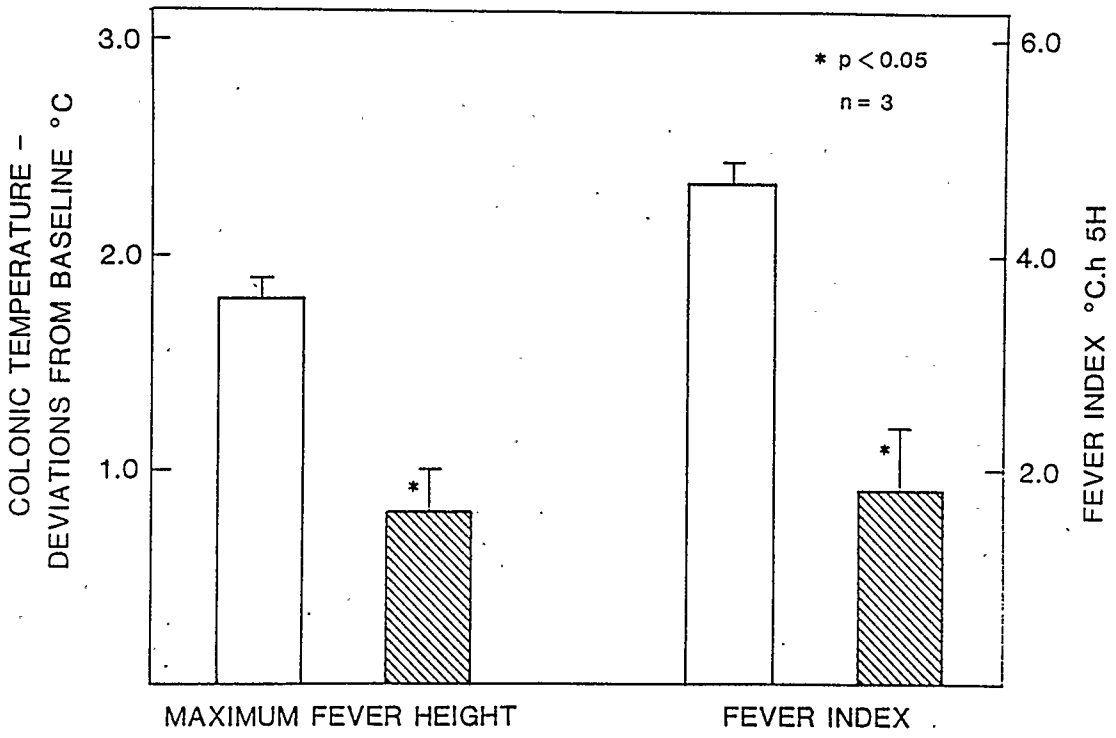



FIGURE 6 Effect of AVP on two indicators of the febrile response (± SEM), maximum fever height and fever index, to intracerebroventricular SAE (10 ng). (*p < 0.05).

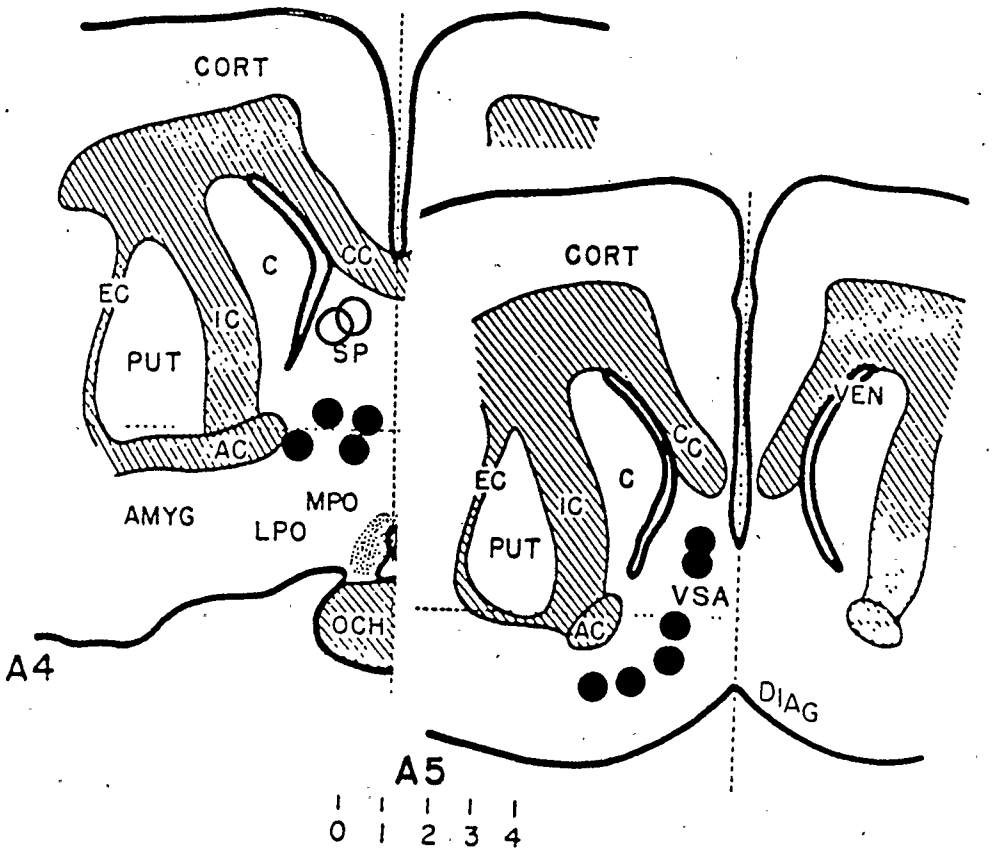



FIGURE 7 Schematic representation of the rabbit brain. Sites at which AVP was effective in producing antipyresis are indicated by closed circles. Sites where no antipyresis was observed are indicated by open circles. Loci from both sites are represented on the one side.
Abbreviations: AC-Anterior Commissure; AMYG-Amygdala; C-Caudate; CC-Corpus Callosum; CORT-Cortex; DIAG-diagonal band of Broca; EC-External Capsule; IC-Internal Capsule; LPO-Lateral Preoptic Area; MPO-Medial Preoptic Area; OCH-Optic Chiasm; POA-Preoptic Area; PUT-Putamen; SP-Septum Pellucidum; VEN-Ventricle; VSA-Ventral Septal Area.

fever. These sites are in the more ventral aspects of the septum, just lateral to or within the distribution of the diagonal bands of Broca.

Another group of four animals was tested to determine whether the icv administration of AVP could suppress a bacterial pyrogen fever. As indicated in Figure 8 (upper panel), there was no significant difference ($p > 0.05$) in the maximum fever height or the fever index observed after intravenous bacterial pyrogen in the absence or presence of 5.0 μg/10 μl of AVP injected intraventricularly. In addition, icv administration of this concentration of AVP was without effect on the body temperature of afebrile rabbits (lower panel).

4) Discussion

The results of the experiment described here demonstrate that AVP does have antipyretic activity in the rabbit in a manner similar to that described already for this peptide in the sheep (Kasting et al., 1979a). When AVP is perfused within the ventral septum of the rabbit there is a marked attenuation of the fever typically evoked by administration of a bacterial pyrogen. The antipyretic action of AVP is observed after both intravenous and intracerebroventricular administration of the bacterial pyrogen. Thus, it is clear that central administration of this peptide has fever reducing properties against both peripherally and centrally administered pyrogens. The reduction in fever observed with AVP is not due to a non-specific decrease in body temperature since perfusion of AVP alone within the VSA had no effect on the normal body temperature of these animals.

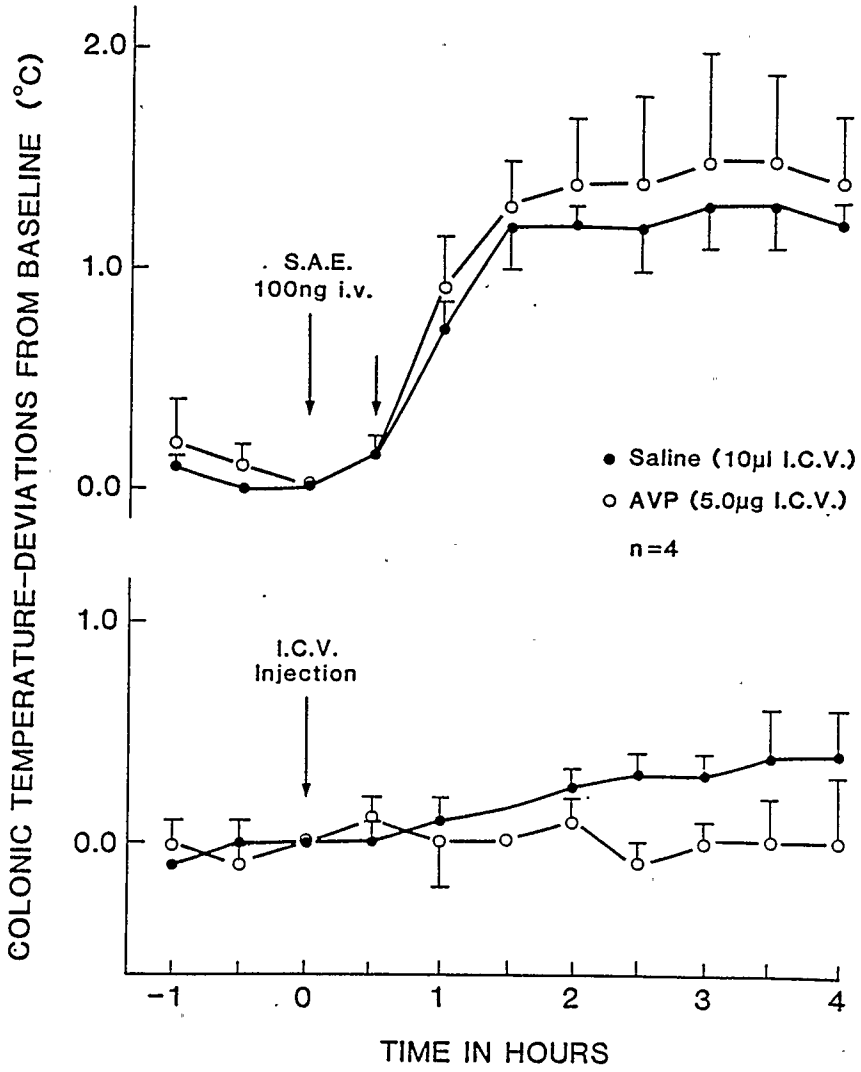



FIGURE 8 Upper panel: mean temperature responses (± SEM) evoked by an intravenous injection of SAE at time zero (first arrow). AVP (5.0 µg/10µl) or the vehicle alone (10 µl sterile saline) was infused into a lateral cerebral ventricle 30 minutes after the injection of the pyrogen (second arrow). Lower panel: mean temperature responses (± SEM) observed after icv administration of AVP (5.0 µg/10 µl), or the saline vehicle (10 µl) into afebrile rabbits.

Further, AVP appears not to alter the ability of the rabbit to conserve heat since the peptide did not alter vasoconstrictor responses to pyrogen when compared to control values.

An estimate of the amount of AVP reaching the tissue can be made based on the observation that there is an exchange of about 10% (Veale, 1971) or less (personal observation) of the perfusion solution in the perfusate with the tissue. Consequently, at the dose of AVP used, a total of 2.7 µg per side would be delivered over a 3 hour perfusion period. This concentration of AVP corresponds closely to that used by previous investigators who have examined the putative role AVP plays in modulating the febrile response (Bernardini et al., 1983). In accordance with this report concerning the antipyretic action of AVP in the rabbit (Bernardini et al., 1983), an icv injection of vasopressin was found to be ineffective in reducing fever. However, in contrast to this previous report, fever was not enhanced after icv vasopressin and the mild hypothermia reported after icv infusion of the peptide (Bernardini et al., 1983; Lipton and Glyn, 1980) was not observed. The reasons for such a discrepancy are not clear at present.

That AVP has an antipyretic action when perfused directly with the VSA, but not when it is infused into the ventricles, does not represent a unique situation. Indeed, a similar response has been observed in the Golden Hamster in which flank marking behaviour is triggered by a direct microinjection of vasopressin into the medial hypothalamus but not by ventricularly administered AVP (Ferris et al., 1984). These data indicate that specific physiological roles for

neuropeptides are unlikely to be determined if only the ventricular route of administration is utilized to investigate their actions.

The sites of maximum sensitivity to the antipyretic effect of AVP are located within the ventral area of the septum, proximal to the diagonal band of Broca (see Fig. 7). This may explain why Bernardini et al. (1983) were unable to induce antipyresis with intraseptal microinjections of AVP since their injections were located in much more dorsal aspects of the septal area. In another species, the rat, this dorsal component of the septal area has been shown to have little or no AVP-sensitive antipyretic actions (see later). An additional aspect of the work reported herein is the use of the push-pull perfusion technique which permits a constant, small amount of peptide to be delivered to the tissue over a long period of time - something that is not possible with a single bolus microinjection.

The hypothesis that AVP may function as a neurotransmitter in a VSA system which is involved in the control of core temperature (Veale et al., 1981) is supported by the presence of dense networks of fibres immunoreactive for vasopressin in this area (Buijs et al., 1978; Sofroniew, 1983; Swanson, 1977; De Vries et al., 1985). The manner in which AVP might exert its antipyretic action is not clear but it may occur as a result of the activation of a neuronal pathway which evokes the release of AVP from terminals in the VSA during fever. An alternative, as yet untested hypothesis, is that AVP might exerts its effect indirectly via an action on some other neuroactive substance. This might explain why in some cases the antipyretic action of AVP was not immediate.

Thus, further evidence is provided to support the hypothesis that AVP may act as an endogenous antipyretic within the mammalian brain. Perfusion of vasopressin within the VSA suppresses fever in rabbits when endotoxin is administered either systemically or centrally. The sites at which antipyresis can be obtained are similar to those already described for both the sheep and rat. It is possible that the differences in sites and method of peptide administration (i.e., push-pull perfusion versus single bolus microinjection) are the reasons for the inconsistent findings concerning the antipyretic actions of AVP in the rabbit. Future studies will focus on the nature of the complex schema which has been proposed to describe the pathogenesis of fever and will attempt to determine the point at which AVP acts to attenuate fever. Moreover, the precise neuroanatomical locus in which AVP is effective requires further pharmacological investigation.

## B. EFFECTS OF INTRASEPTAL HUMAN PITUITARY GLYCOPEPTIDE ON ENDOTOXIN FEVER

### 1) Introduction

Human pituitary glycopeptide (HPGP) is a 39 amino acid residue with an oligosaccharide chain (Seidah et al., 1981) isolated recently from the human pituitary gland. This novel glycopeptide is believed to represent the glycopeptide fragment of pro-neurophysin-arginine vasopressin (Seidah et al., 1981) since it is localized in the hypothalamus and pituitary of rats (Lu et al., 1982) in a pattern similar to that described for the distribution of vasopressin, oxytocin and neurophysins (Zimmerman et al., 1974). Currently, the biological importance (if any) of HPGP is unknown. This study was carried out to determine the thermoregulatory actions of this peptide. Specifically, to determine whether, like vasopressin, HPGP could suppress pyrogen fever when perfused within the ventral septum.

### 2) Methods

Surgical procedures, injections, push-pull perfusions, histological verification and temperature measurements were similar to those described earlier. In addition, human pituitary glycopeptide (supplied by M. Chretien) was dissolved in sterile aCSF.

3) Results

Figure 9 shows both the colonic and earskin temperatures during perfusion of the VSA with HPGP at a concentration of 13.0 µg/ml and a perfusion rate of 30 µl/min. Perfusion of HPGP within the VSA resulted in a long latency rise in core temperature which was not accompanied by earskin vasoconstriction. Perfusion of HPGP in the VSA during fever altered the normal febrile response (Figure 10). The fever onset was delayed and the maximum fever height was greater in the rabbits that received HPGP. In contrast, during perfusion with aCSF, a characteristic biphasic fever was evoked which was accompanied by earskin vasoconstriction.

4) Discussion

Perfusion of HPGP within the VSA evoked a rise in core temperature which was not associated with peripheral vasoconstriction. When HPGP was perfused similarly following icv endotoxin, a fever was evoked which was suppressed initially but which proceeded rapidly to higher than control levels after 1.0 hour. The rise in temperature observed here corresponded to when HPGP elevated body temperature in the afebrile rabbit indicating that the exaggerated fevers may be due to an hyperthermic action of HPGP superimposed on the febrile effect of icv endotoxin. The febrile response was not accompanied by

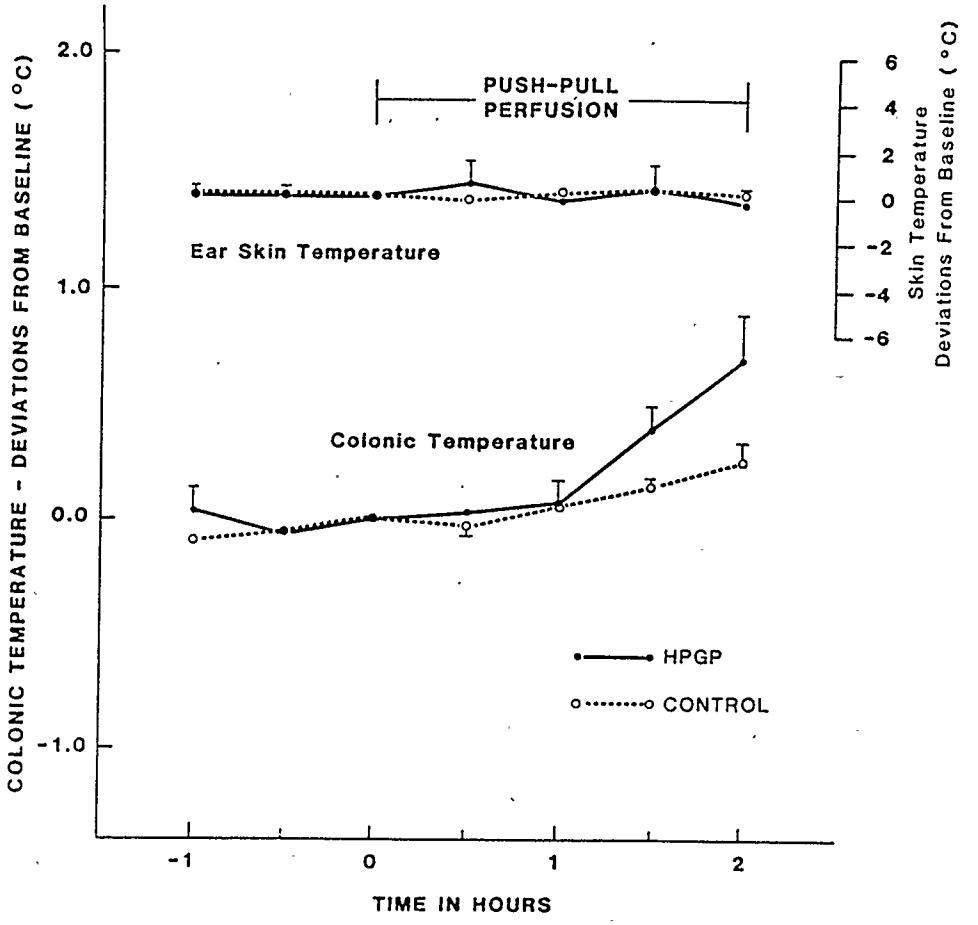



FIGURE 9 Mean colonic and earskin temperature responses (± SEM) observed in 3 rabbits. HPGP at a concentration of 13.0 μg/ml (closed circles) or aCSF (open circles) was perfused within the VSA of the afebrile rabbit.

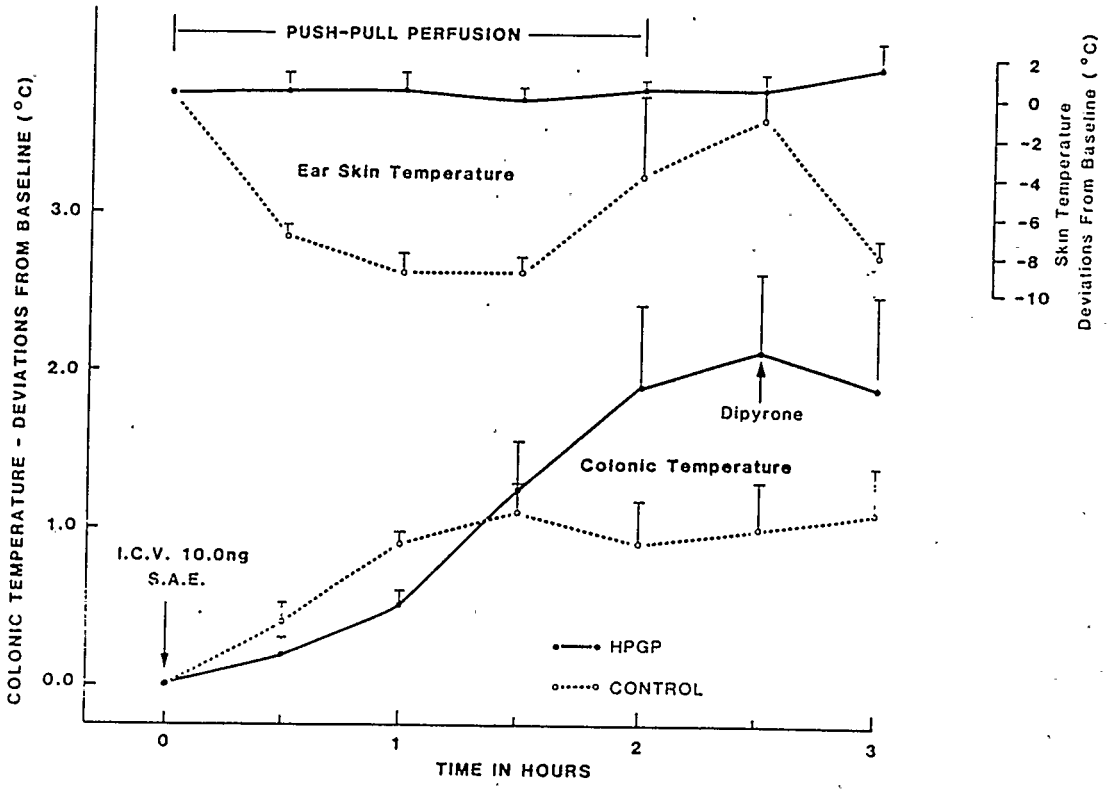



FIGURE 10 Mean colonic and earskin temperature responses (± SEM) observed in 3 rabbits. HPGP at a concentration of 13.0 µg/ml (closed circles) or aCSF (open circles) was perfused in the VSA immediately prior to and for 2.0 h following an intracerebroventricular injection of SAE (10 ng) at time zero.

vasoconstriction but rather by vigorous, whole body shivering. Therefore, if HPGP exhibited any antipyretic properties these were masked by an action of the peptide to recruit heat gain effectors to raise body temperature. Indeed, the body temperature of the HPGP treated rabbits rose sufficiently high to warrant antipyretic treatment with dipyrone. It is conceivable that the sample of HPGP used in this study was contaminated with endotoxin and it was this that evoked the hyperpyrexia observed under these conditions. However, the lack of peripheral vasoconstriction during the febrile episode makes this possibility unlikely.

Although these experiments opened up some intriguing possibilities, they were discontinued owing to the lack of HPGP and the fact that following the large fevers, 2 out of 3 animals died.

# III. ANTIPYRETIC ACTION OF VASOPRESSIN IN THE RAT

## A. EFFECTS OF VASOPRESSIN ON PROSTAGLANDIN-$E_2$ INDUCED HYPERTHERMIA

### 1) Introduction

Although the exact neurochemical mechanisms underlying the development of fever have yet to be clearly established, it is believed that prostaglandins of the E series may play an integral role in this process (Milton and Wendlandt, 1971). Current evidence indicates that these metabolites of arachidonic acid act primarily within the rostral diencephalon (Feldberg and Saxena, 1971; Veale and Cooper, 1975). In the rat, an extensive exploration of prostaglandin sensitive hyperthermic sites indicates that the region of maximum sensitivity extends from the nuclear areas just rostral to the posterior hypothalamus and continues toward the diagonal bands of Broca (Williams et al., 1977). Initial indications are that AVP may act within the ventral septal/diagonal band of Broca area of the sheep (Cooper et al., 1979) and rabbit brain (see earlier) to suppress fever. This area is located in the rostral regions of the anterior hypothalamic, preoptic area (PO/AH) and extends anterior toward the diagonal bands of Broca, just ventral to the septum. Therefore, the area corresponding to these AVP-sensitive sites also is remarkably sensitive to prostaglandins (Williams et al., 1977).

This work was undertaken to delineate further the site and mechanism of action of AVP in the negative modulation of fever (Kasting et al., 1982). Specifically, the action of AVP in

suppressing a hyperthermia evoked by $PGE_2$ (a putative mediator of fever) was examined in the rat by perfusing the peptide in sites considered to be both prostaglandin and vasopressin sensitive.

2) Methods

Male Long Evans rats, weighing from 285 to 325 g were housed in a colony room maintained at 23-25°C and illuminated on a 12-hr light-dark cycle. For surgery, each rat was anaesthetized with 65 to 75 mg/kg sodium pentobarbital, injected into the peritoneal cavity. Using the stereotaxic coordinate system of Paxinos and Watson (1982), an array of three 20 ga stainless steel guide tubes was implanted. The guide tubes were lowered so that the tips rested 2.0 to 4.0 mm above the intended injection/perfusion sites. Two of the guides were implanted bilaterally above the ventral septal area (0.3 mm anterior to bregma; 1.0 mm lateral to the midline and 3.0 mm below dura). The third guide tube was implanted just above the right or left lateral cerebral ventricle (1.3 mm caudal to bregma; 1.75 mm lateral to the midline and 3.0 mm below dura). Following insertion of three anchor screws into the calvaria, the cannulae were secured in place with cranioplastic cement. Each guide tube was fitted with an indwelling 23 ga stylet of corresponding length, both of which were bevelled at a 45° angle.

To microinject $PGE_2$ (Upjohn) or saline directly into the VSA, a 30 ga thin-walled stainless steel injector needle was lowered through the guide tube to a predetermined depth. The needle was connected by a length of PE-20 tubing, filled with the solution to be injected, to

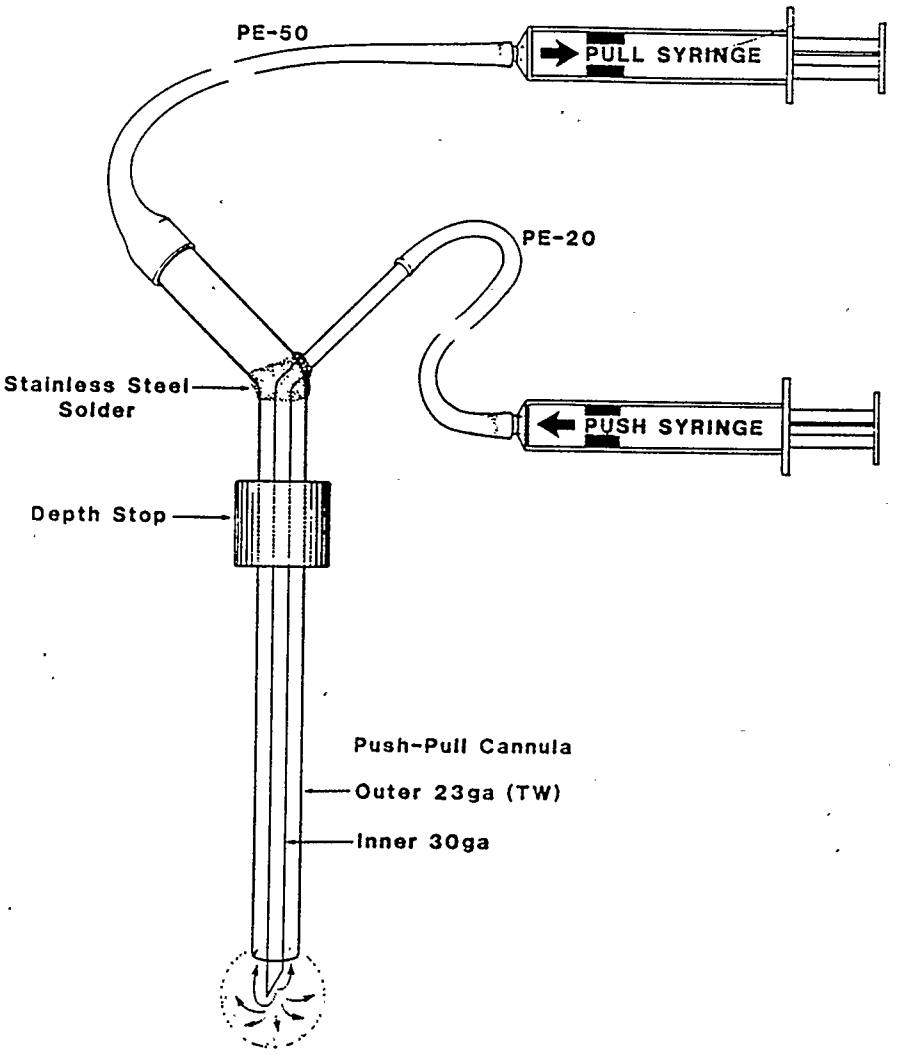



FIGURE 11 Diagram of push-pull cannula used for smaller animals like rats. The small size allows bilateral perfusions to be undertaken in sites close to each other.

a Hamilton 2.5 ml syringe which was mounted on a Harvard infusion pump. The microinjection of $PGE_2$ or saline was made in a volume of 0.5 µl over a period of 30-60 seconds. The prostaglandin solution was prepared in a pyrogen free 0.9% saline solution (Travenol).

A YSI 701 thermistor probe was inserted 6 cm beyond the anus and held in place by surgical tape wrapped gently around the tail of the conscious and unrestrained rat. Colonic temperature was monitored and recorded simultaneously on a Digitec Datalogger. The baseline temperature of each rat was obtained for at least 60 minutes before an experiment began and at 5-15 min intervals thereafter until completion of the experiment.

To perfuse arginine vasopressin (Bachem; 6.5 µg/ml) or artificial cerebrospinal fluid (aCSF; Schwartzkroin, 1975), within the ventral septal area of the brain, a modified push-pull cannuala system was employed (Figure 11). The bevelled tip of the push cannula was extended 0.5-1.0 mm beyond the tip of the pull cannula. With this separation of the cannulae, it is possible to bathe a sphere of tissue approximately 1.0-1.5 mm in diameter (Myers, 1971). A Harvard infusion withdrawal pump had been calibrated previously to deliver 16 µl over a 1 min period. AVP was prepared immediately prior to each experiment. The stock solution of AVP was initially diluted in 0.025 M acetic acid. Thereafter, each aliquot of AVP could be diluted to the appropriate concentration with sterile aCSF.

The $PGE_2$ sensitive loci in the VSA were perfused bilaterally for 120 min prior to an injection of 2.0 µg/10 µl of $PGE_2$ into the lateral cerebral ventricle and for an additional 120 min after the icv

injection. The sequence of perfusion of AVP or the vehicle alone was randomized prior to experimentation.

Upon completion of the experiments, each rat was anaesthetized deeply with sodium pentobarital. After the heart was clamped, the brain was perfused with saline followed by formalin. The brain was blocked in the coronal plane and sectioned at 50 μm on a sledge microtome. Each section was stained and the sites localized by light microscopy.

Data were analyzed according to Student's t distribution for paired subjects. A fever index was computed for each response. That is the area under the temperature time curve was expressed as °C.h for 1.5 hours after injection of the prostaglandin.

3) Results

Prostaglandin-sensitive sites were localized in each animal by direct administration of $PGE_2$ into the ventral septum. Following microinjection of 0.5 μl of sterile saline, 100 ng of $PGE_2$ in the same volume were injected bilaterally at least 60 min later into discrete sites in the ventral septal area of the rat. Figure 12a indicates that sterile saline had no effect on body temperature whereas prostaglandin $E_2$ evoked a rapid and intense hyperthermic response. The maximum increase in body temperature was achieved within 60 min after injection. Figure 12b shows that body temperature increased to nearly $2^{o}C$ above baseline body temperature ($p < 0.001$ compared with saline).

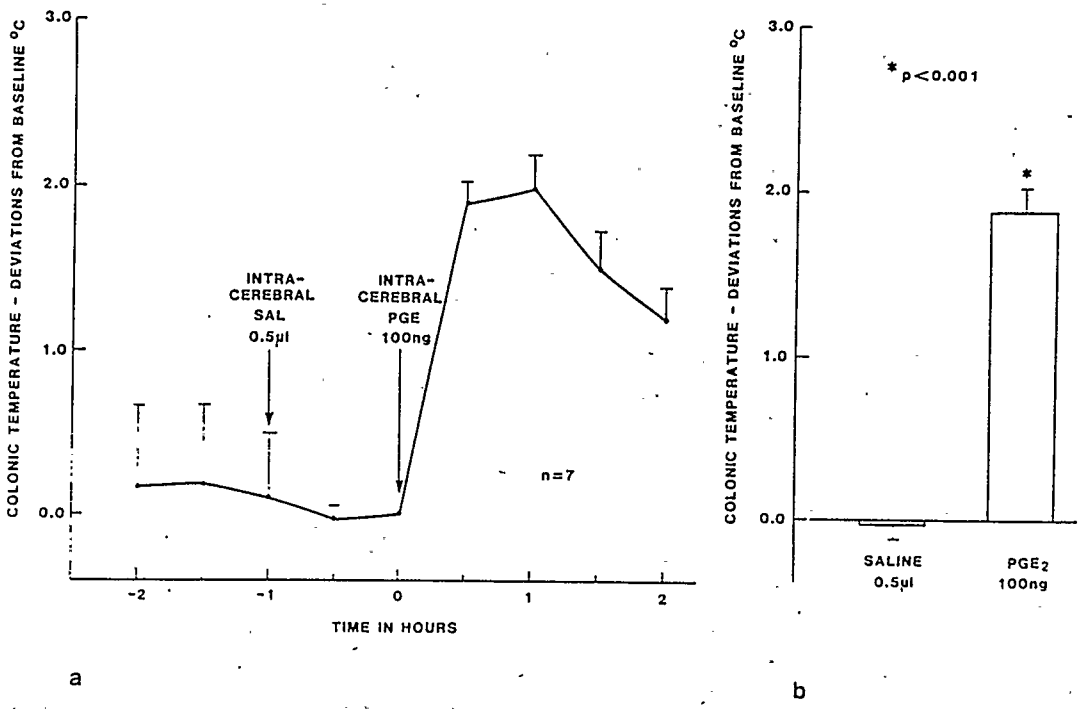



FIGURE 12 a) Mean temperature response (± SEM) observed in 7 animals to direct intracerebral microinjection of sterile saline and $PGE_2$. At time 0, 100 ng/0.5 µl of $PGE_2$ was injected bilaterally into the ventral septal area. At -1, a similar microinjection of sterile saline was made.
b) Absolute magnitude of the temperature increase after 30 min. The hyperthermia evoked by $PGE_2$ was significantly different from that evoked by a control injection of saline ($^{*}p < 0.001$.).

Perfusion of AVP within these prostaglandin-sensitive sites markedly altered the hyperthermic response to an intracerebroventricular (icv) injection of $PGE_2$. When aCSF was perfused within these ventral septal sites, an icv injection of 2.0 µg of $PGE_2$ in 10 µl evoked an increase in core temperature of 1°C. However, when the icv injection of $PGE_2$ was made during perfusion of this area with AVP (6.5 µg/ml), there was little or no increase in body temperature. Illustrated in the upper panel of Figure 13 is the temperature response in an individual animal in which the sites were perfused initially with aCSF and then with AVP. The bottom panel of Figure 13 illustrates the suppressive action of the peptide against $PGE_2$ hyperthermia when the sites were perfused initially with AVP and subsequently with the carrier vehicle (aCSF) 1-2 days later. The suppression of the hyperthermic response to $PGE_2$ occurred independently of the order of administration of arginine vasopressin and aCSF. Figure 14 illustrates the mean temperature responses of 6 animals. The temperature response to $PGE_2$ was suppressed significantly for up to 90 minutes after icv administration of this hyperthermic agent. In every case, core temperature of the animal returned to normal within two hours following the intracerebroventricular injection. Neither perfusion of AVP into the ventral septal area nor intracerebroventricular injection of saline (10 µl) had a significant effect on resting body temperature.

The absolute magnitude of the hyperthermia and the fever index were both significantly reduced after administration of AVP. Figure 15a indicates the average increase in core temperature for 6 animals. When $PGE_2$ was administered during perfusion of aCSF the absolute

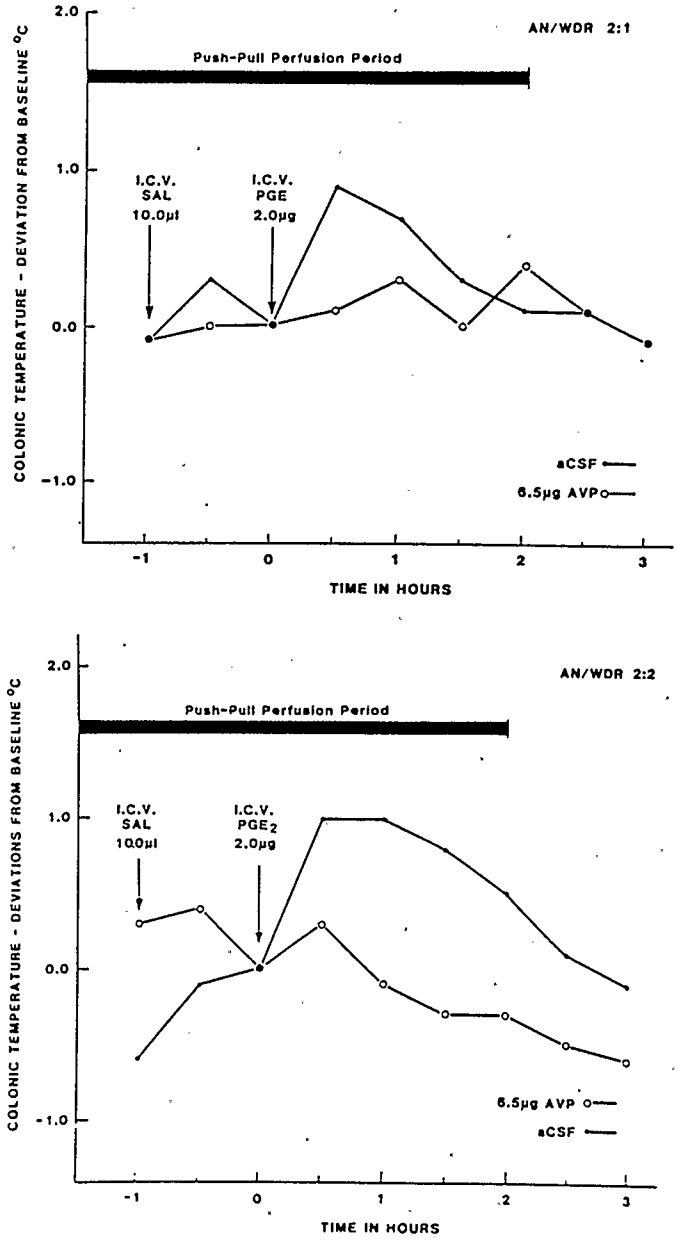



FIGURE 13 Suppression of $PGE_2$ hyperthermia by AVP perfused bilaterally into the VSA. (Top) Initial perfusion of aCSF was without effect on the hyperthermic response evoked by 2.0 µg of $PGE_2$ injected icv in an individual rat. Subsequent perfusion with 6.5 µg/ml AVP prevented the increase in core temperature evoked by $PGE_2$. (Bottom) Similar response observed in another animal in which the order of perfusion was reversed.

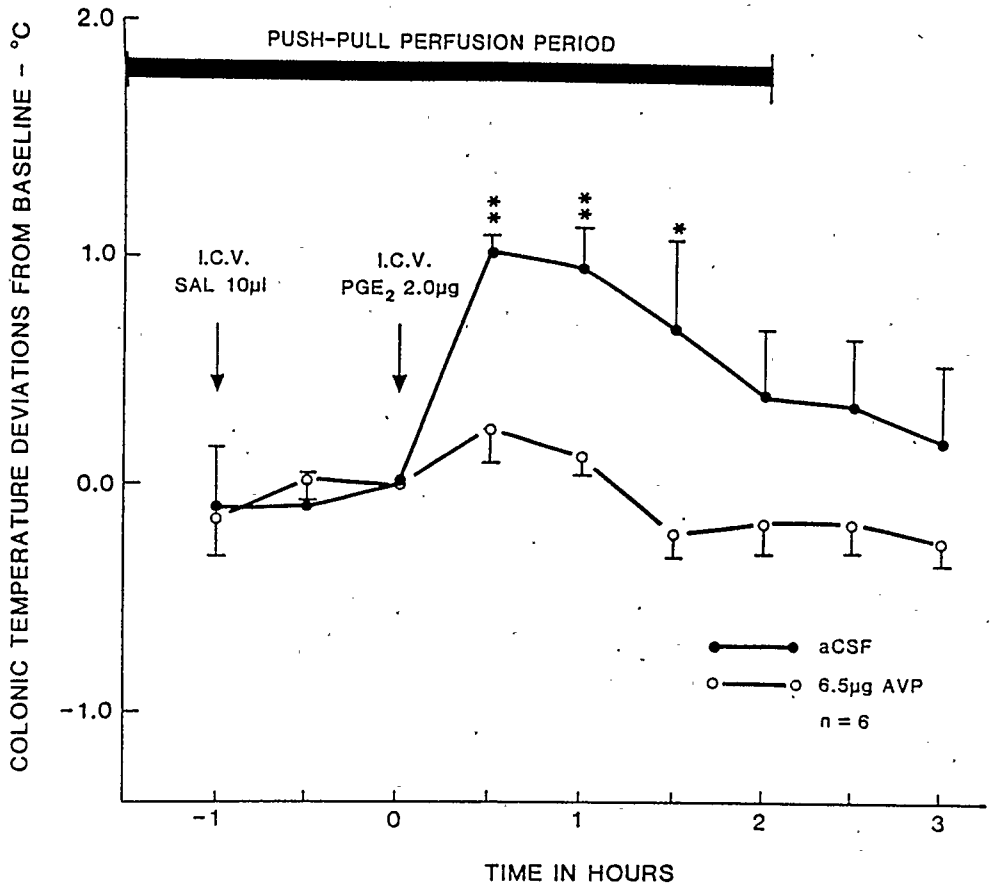



FIGURE 14 Mean colonic temperature responses (± SEM) observed in 6 animals. Perfusion of 6.5 µg/ml AVP suppressed the hyperthermic response to icv $PGE_2$. Similar perfusion with aCSF was without effect on the $PGE_2$-induced hyperthermia. (** $p < 0.01$; * $p < 0.05$).

magnitude attained was over 1.0°C. Conversely, when $PGE_2$ was injected into the lateral cerebral ventricle during perfusion with AVP there was a significant ($p < 0.01$) attenuation of the hyperthermia, less than 0.5°C in 30 minutes. Figure 15b illustrates the fever indices calculated for $PGE_2$ under each of these two conditions. Again, the response to $PGE_2$ in the presence of aCSF was significantly greater ($p < 0.01$) than that obtained when the $PGE_2$ was administered in the presence of vasopressin.

Histological verification of the sites of perfusion indicated that the region of greatest sensitivity to AVP was located in the VSA. Sites at which perfusion of this peptide was ineffective in suppressing the $PGE_2$-induced hyperthermia were located more dorsally within the lateral septum. When AVP was perfused in these sites the hyperthermic response to icv $PGE_2$ was similar to that evoked by $PGE_2$ during perfusion with aCSF. The spheres presented in Figure 16 represent the locus of perfusion.

4) Discussion

It is believed that during the initial stages of fever, cells of the reticulo-endothelial system release endogenous pyrogen into the circulation (Dinarello, 1979). It is believed further that this polypeptide acts on cells of the rostral diencephalon through a process which is mediated, at least in part, by prostaglandins of the E series (Milton, 1982). Within this same region of the brain AVP has been shown to be effective in reducing fevers of bacterial origin (Cooper et al., 1979; Kasting et al., 1979a, see earlier). Thus,

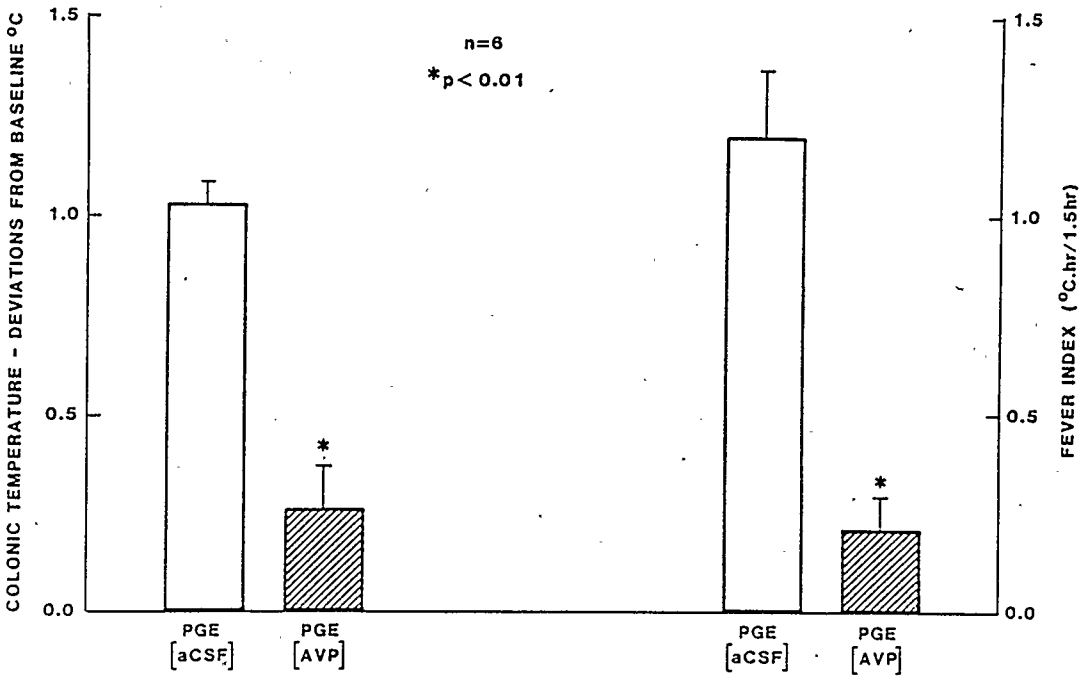



FIGURE 15 AVP alters the characteristics of the hyperthermic response to $PGE_2$. Perfusion of AVP within the ventral septal area significantly reduced the absolute magnitude (± SEM) of the hyperthermia; (aCSF = 1.03 ± 0.06; AVP = 0.26 ± 0.11). The fever index (± SEM) over 90 minutes was similarly reduced during perfusion with AVP (aCSF = 1.20 ± 0.17; AVP = 0.21 ± 0.08). The AVP induced reductions were significantly different from controls ($p < 0.01$).

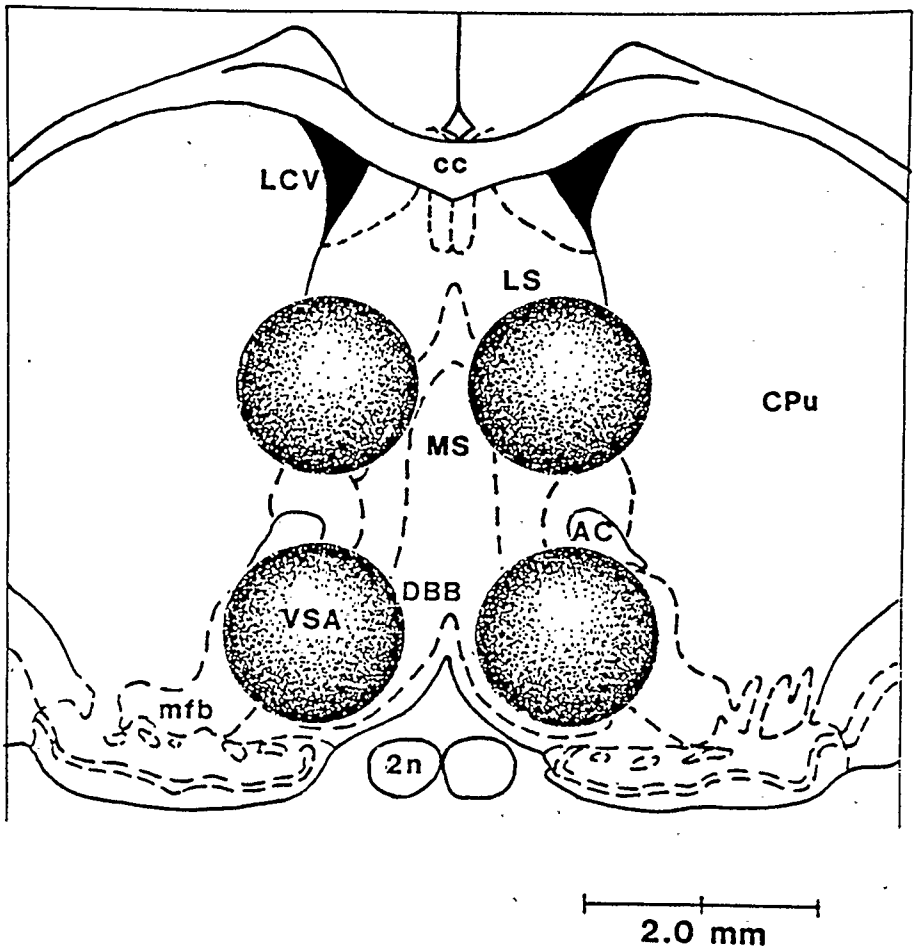



FIGURE 16 Schematic histological section. Sites at which AVP was effective in attenuating $PGE_2$ hyperthermia are located in the ventral septal area (VSA), sites at which this peptide was ineffective are also indicated (lateral septal area).

Abbreviations: AC - Anterior Commissure; cc - corpus callosum; CPu - caudate putamen; DBB - diagnonal band of Broca; LCV - lateral cerebral ventricle; LS - lateral septal nucleus; mfb - medial forebrain bundle; MS - medial septal nucleus; 2n - optic nerve; VSA - ventral septal area.

although a possible site of action for AVP has been postulated, the mechanism by which vasopressin exerts its antipyretic response has not been determined. To determine more precisely the mechanism of action, the effect of AVP on one potential mediator of the febrile response, $PGE_2$, was examined.

Prostaglandin-sensitive sites were located lateral to the vertical limb of the diagonal band of Broca, immediately ventral to the septum, a finding consistent with previous reports regarding the neuroanatomical loci of PGE-sensitive sites in the rat brain (Williams et al., 1977). More recently, Sofroniew (1983) and De Vries et al. (1985) have demonstrated the existence of neurons containing AVP within the medio-lateral septum and also within the nucleus of the diagonal band, an area which corresponds closely to where vasopressin exerted its antipyretic action in this study. The presence of AVP along with specific receptor binding sites (Baskin et al., 1983) in the VSA are supportive of a neurotransmitter role for AVP in this area of the brain. Thus, perfusion of AVP within the VSA is as effective in preventing the hyperthermic response to $PGE_2$ as it is in preventing fever of bacterial pyrogen origin. This antipyretic action was achieved with doses which are comparable to those previously reported to be effective against pyrogen fever. Animals that received AVP did not manifest the physiological changes normally associated with fever, including huddled posture, slowed respiratory rate or blanching of the skin. These physiological changes did occur in animals during experiments in which pyrogen was given and the control vehicle solution was perfused.

After icv injection of $PGE_2$, during push-pull perfusion of the VSA with aCSF, the typical rapid increase in body temperature was observed. This response was followed by an equally rapid defervescence, a characteristic of this route of administration. This observation suggests that the push-pull perfusion technique per se does not interfere with either the development or termination of the hyperthermic response. Similarly, perfusion with AVP and the vehicle, or with the vehicle alone, did not have any significant effects on resting body temperature suggesting that the observed reduction in the prostaglandin hyperthermia was not due solely to a nonspecific decrease in normal body temperature as may be the case with some potent thermolytic peptides (Kasting, 1980).

Although the present results support an interaction between AVP and prostaglandins in modulating fever, the precise nature of such an interaction cannot as yet be specified with certainty. It may be that AVP acts directly to disrupt the events which are mediated by the release of prostaglandins during the development of fever. Alternatively, vasopressin may be affecting another component of the system which, in turn, indirectly influences the processes to which the prostaglandins are integral. Presently, it is possible only to conclude that AVP does prevent the hyperthermia caused by both of the pyrogenic agents, bacterial pyrogen and prostaglandins of the E series. Such observations may serve to define more clearly the means by which AVP contributes to antipyresis and the prevention of potentially lethal hyperpyrexias.

# IV. CHARACTERIZATION OF THERMOREGULATORY AND CONVULSIVE ACTIONS OF VASOPRESSIN

## A. THERMOREGULATORY ACTIONS OF CENTRALLY ADMINISTERED VASOPRESSIN IN THE RAT

### 1) Introduction

When AVP is infused into the lateral cerebral ventricle of the rat a hypothermia of 0.5 - 1.5°C is elicited within 5 - 15 minutes after the infusion (Kasting et al., 1980; Meisenberg and Simmons 1984). In addition, perfusion of vasopressin into sites within the VSA suppresses fever evoked by peripheral administration of a pyrogen (Kasting et al., 1979a), central administration of a pyrogen (see earlier) or prostaglandin $E_2$ (see earlier). In apparent contrast to these findings, a recent report indicates that microinjection of AVP into discrete sites in the PO/AH evokes a dose-related increase in core temperature in cool, neutral and warm environments, perhaps indicative of a shift in the set-point for body temperature control (Lin et al., 1983). Such differences in responses have been used to question the generality of an antipyretic role for endogenous (and exogenous) AVP (Lipton and Clark, 1986).

Is it possible that AVP acts centrally to mediate three such disparate systems as: 1) heat dissipation, 2) antipyresis and (3) heat-conservation/production? To understand the role AVP plays in thermoregulation and fever more fully, it is important to ascertain whether the hypothermic action of the peptide can be localized to a

specific site in the brain. It also is important to determine whether the increase in core temperature seen following direct intracerebral administration is a specific action mediated by an AVP receptor or, perhaps, to contamination by bacterial pyrogens or a non-specific release of prostaglandins due to mechanical stimulation of brain tissue (Rudy et al., 1977).

These series of experiments were conducted: 1) to elucidate more fully the thermoregulatory actions of AVP; 2) to determine the neuroanatomical locus of these actions; and 3) to ascertain whether such actions are mediated by an AVP receptor.

2) Methods

Fifty-eight male Long Evans rats (0.280 - 0.350 kg) were used for the experiments. Animals were maintained on a 12 hour light/dark cycle at an environmental temperature of 25 ± 3°C. Each animal had ad libitum access to water and standard laboratory rat chow.

Under barbiturate-induced anaesthesia (65 mg/kg ip), bilateral 20 ga (thin wall) stainless steel guide tubes were implanted stereotaxically (Paxinos and Watson, 1982) so that the tips rested 3 - 5 mm above the intended site of injection in the nucleus accumbens (NA; n = 6), ventral septal area (VSA; n = 22), substantia innominata (SI; n = 6), preoptic area (POA; n = 8), anterior hypothalamus (AH; n = 7) and dorsomedial hypothalamus (DMH; n = 5). In addition, a single guide cannula was implanted above a lateral cerebral ventricle (LCV; n = 4). Animals were allowed to recover from surgery for a period of 7 days.

Each rat was placed individually in a plastic cage for at least 60 min before microinjection of the peptide or control solution (artificial cerebrospinal fluid; aCSF; Schwartzkroin, 1975). Stock solutions of AVP (Bachem) were diluted initially with 0.025 M acetic acid and maintained at 4°C. Immediately prior to each experiment the peptide was diluted to the appropriate concentration with aCSF. The vasopressin antagonist, $d(CH_2)_5$Tyr(Me)AVP, was diluted similarly with aCSF. The concentration of AVP was 100 ng, whereas that of the antagonist was 200 ng. The volume of injection for each bilateral infusion was 0.5 µl. The peptides were infused into unanaesthetized and unrestrained rats over a 30-45 second period using a 27 ga injector needle affixed by PE-20 tubing to a Harvard infusion pump. For icv infusions, an injector needle was lowered to the appropriate depth and the AVP (1.0 µg/10 µl) was administered by gravity flow. For 1 hour before and 3-6 hours following injection of the peptide or control solution, the colonic temperature of the animal was monitored continuously using a thermistor probe (YSI 701) inserted 8 cm beyond the anus and taped to the tail. The core temperature of each rat was recorded at 5 min intervals on a digital datalogger (United Systems Digitec).

Upon completion of the experiments, each microinjection site was verified histologically. The results were analyzed statistically using Student's t test for paired samples. The fever index was expressed as area under the curve in °C.h.

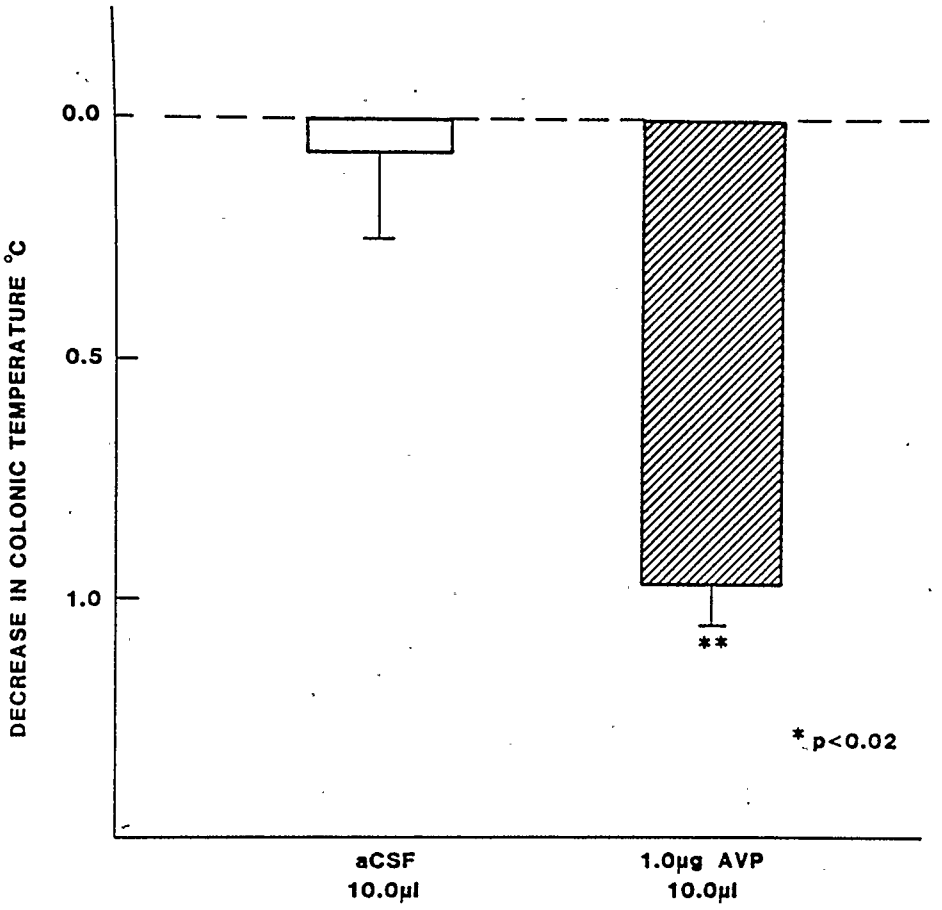


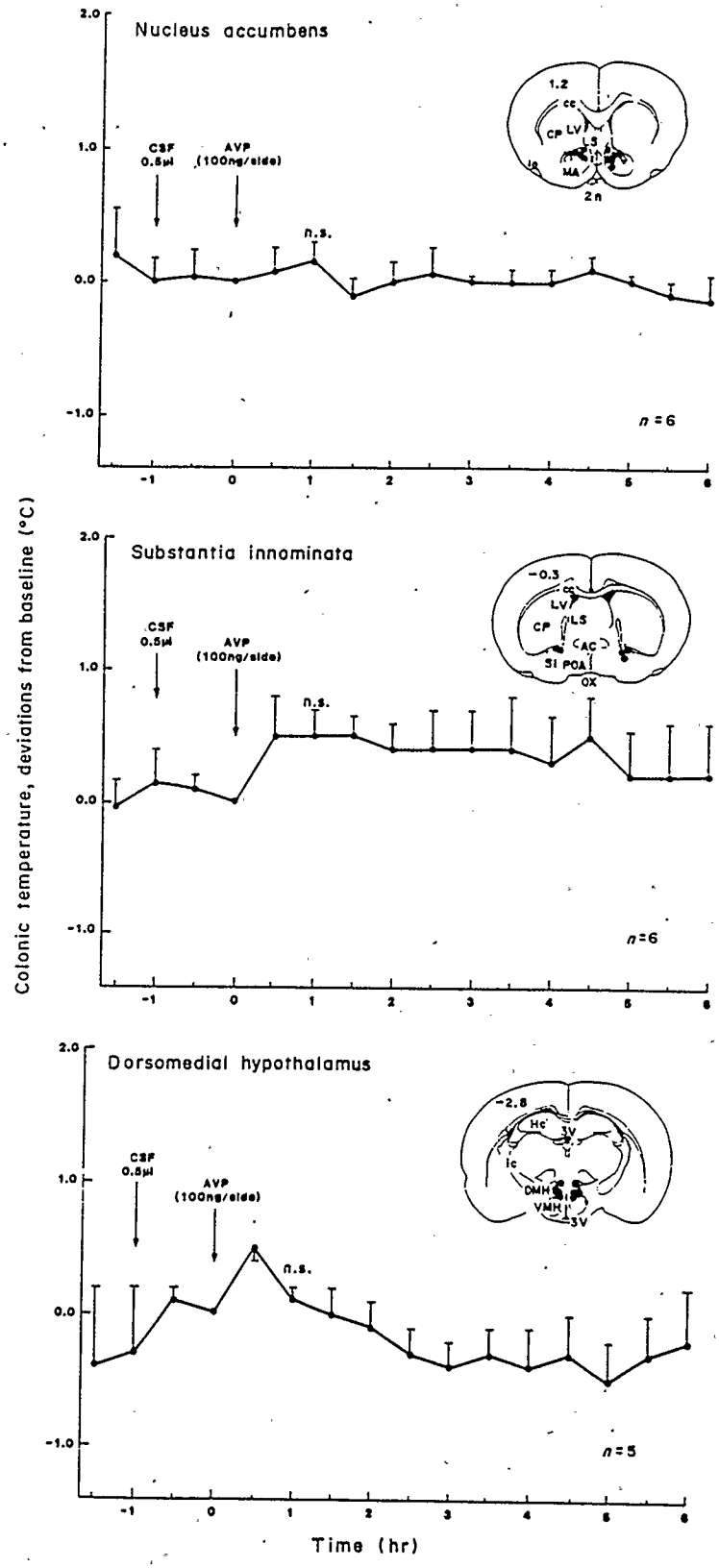

FIGURE 17 Absolute magnitude of the temperature responses (± SEM) observed in 4 rats following intracerebroventricular administration of aCSF (10 µl) or AVP (1.0 µg/10 µl). Maximum hypothermic responses were observed 15 min after infusion. (**p < 0.02 between rats treated with vasopressin and controls given aCSF.)

3) Results

Intracerebroventricular administration of AVP in a dose of 1.0 µg/10 µl evoked a hypothermia of approximately 1°C (Figure 17). This fall in body temperature began within 5 minutes after infusion of the peptide. The core temperature of the animal was at its nadir within 15 minutes following the icv infusion. Within 20-30 minutes after infusion of the AVP, the colonic temperature had returned to baseline levels. Coincident with the hypothermic response, the rats displayed periods of passive immobility and staring. Artificial cerebrospinal fluid was without effect on core temperature.

To specify neuroanatomically those sites at which AVP exerted its action on the thermoregulatory system (indicated by closed circles on the schematic histological sections), 100 ng/0.5 µl/side of the peptide were microinjected into six brain areas. Arginine vasopressin, or the carrier vehicle (aCSF), failed to evoke a significant alteration in core temperature when injected into the NA, SI, and DMH (Figure 18). In addition, when vasopressin was similarly injected into sites within the VSA (an area of the brain in which this peptide exerts antipyretic actions) no significant effects on core temperature were observed in the afebrile rat (Figure 19). However, when AVP was microinjected within the POA, a significant increase in core temperature was observed (Figure 20). The response elicited following injection of AVP into the AH was not significantly different from that of a similar injection of the control solution into this site.

FIGURE 18 Mean temperature responses (± SEM) to direct intracerebral microinjection of aCSF (0.5 µl/side) and AVP 100 ng/0.5 µl/side) into the nucleus accumbens (upper), substantia innominata (middle) and dorsomedial hypothalamus (lower) of the rat. AVP had no significant effects on core temperature of the animals (n.s.). Abbreviations: AC, anterior commissure; cc, corpus collosum; CP, caudate putamen; DMH, dorsomedial hypothalamus; Hc, hippocampus; ic, internal capsule; lo, lateral olfactory tract; LV, lateral ventricle; LS, lateral septum; NA, nucleus accumbens; 2n, optic tracts; OX, optic chiasm; POA, preoptic area; SI, substantia innominata; VMH, ventromedial hypothalamus; 3V, third ventricle.


Nucleus accumbens
CSF 0.5µl
AVP (100ng/side)
n.s.
n=6
Substantia innominata
CSF 0.5µl
AVP (100ng/side)
n.s.
n=6
Dorsomedial hypothalamus
CSF 0.5µl
AVP (100ng/side)
n.s.
n=5
Colonic temperature, deviations from baseline (°C)
Time (hr)

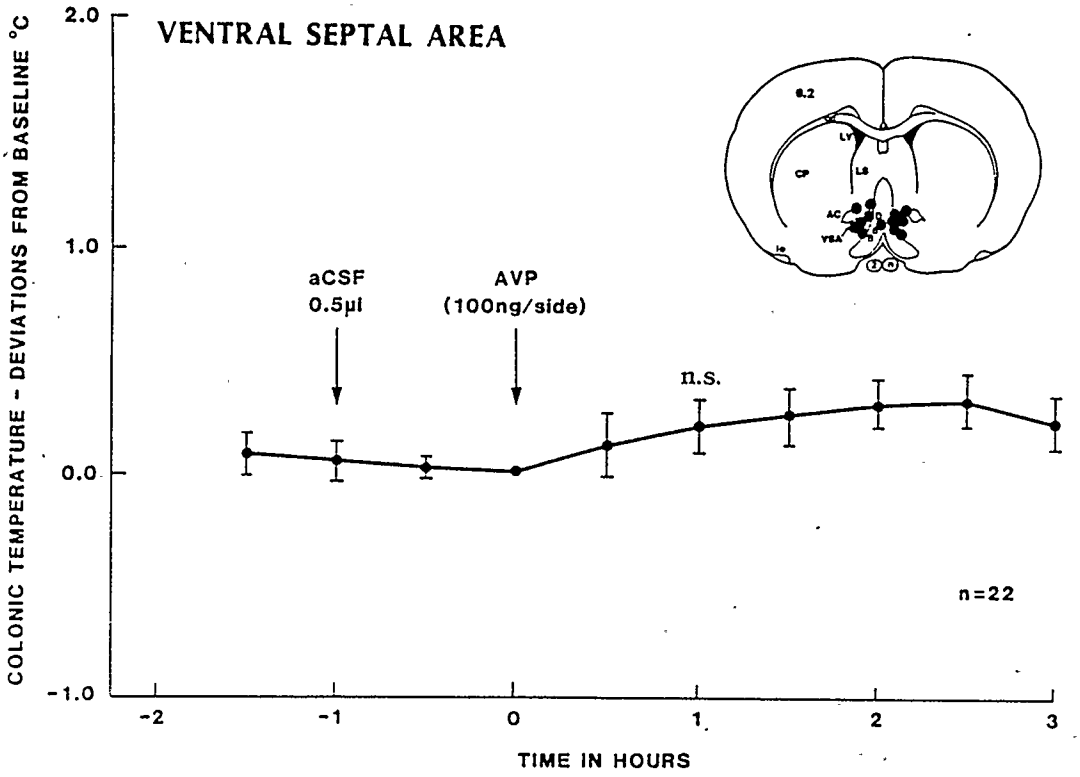
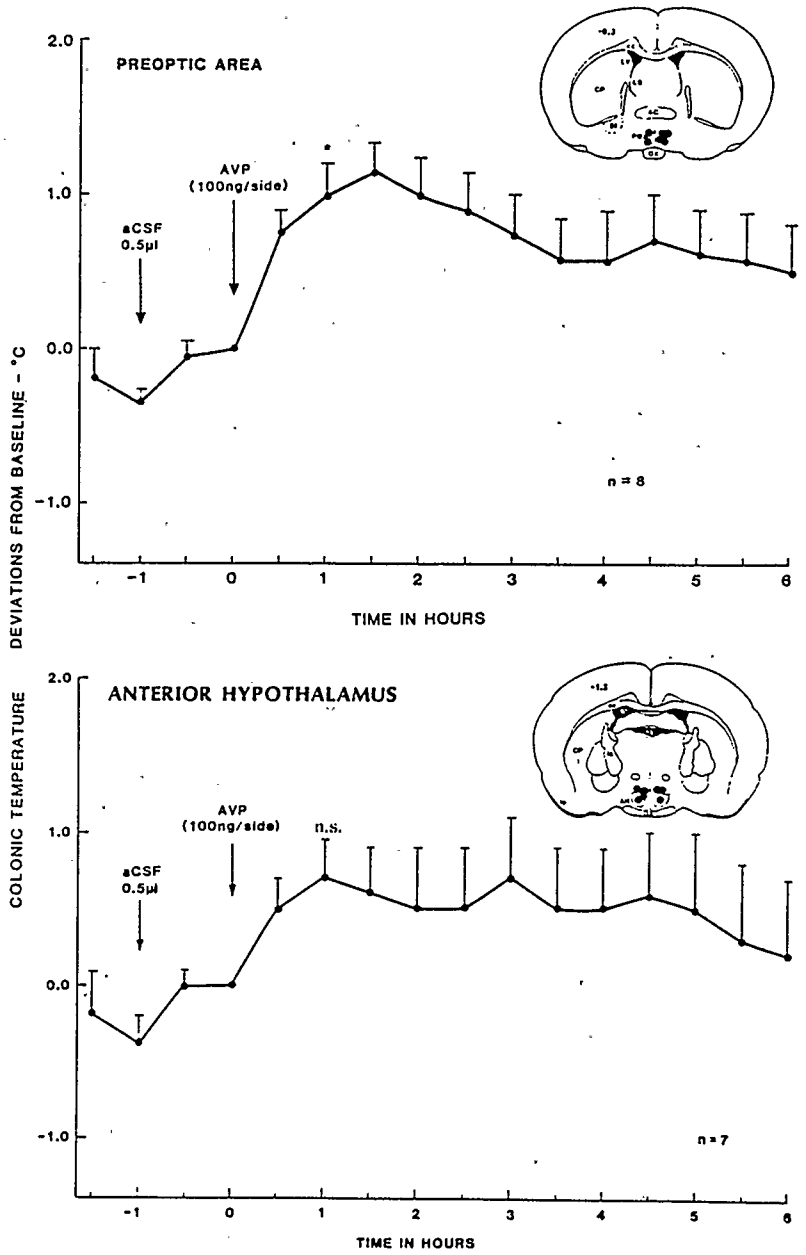



FIGURE 19 Mean temperature response (± SEM) to direct intracerebral microinjection of aCSF (0.5µl/side) and AVP 100 ng/0.5µl/side) into the ventral septal area of the rat. AVP had no significant effects on core temperature of the animals (n.s.). Abbreviations: AC, anterior commissure; cc, corpus callosum; CP, caudate putamen; DBB, diagonal band of Broca; lo, lateral olfactory tract; LS, lateral septum; LV, lateral ventricle; 2n, optic tracts; VSA, ventral septal area.

FIGURE 20 Mean temperature responses (± SEM) to direct intracerebral microinjection of aCSF (0.5 µl/side) and AVP (100 ng/0.5 µl/side) into the preoptic area (upper) and anterior hypothalamus (lower) of the rat. (*$p < 0.01$, 1 hour fever index between rats treated with vasopressin and controls given aCSF; n.s. = no significant difference). Abbreviations: AC, anterior commissure; AH, anterior hypothalamus; cc, corpus callosum; CP, caudate putamen; ic, internal capsule; loc, lateral olfactory tract; LS, lateral septum; LV, lateral ventricle; OX, optic chiasm; POA, preoptic ara; SI, substantia innominata; 3V, third ventricle.


PREOPTIC AREA
aCSF
0.5µl
AVP
(100ng/side)
n = 8
2.0
1.0
0.0
-1.0
DEVIATIONS FROM BASELINE - °C
-1
0
1
2
3
4
5
6
TIME IN HOURS
ANTERIOR HYPOTHALAMUS
aCSF
0.5µl
AVP
(100ng/side)
n.s.
n = 7
COLONIC TEMPERATURE
2.0
1.0
0.0
-1.0
-1
0
1
2
3
4
5
6
TIME IN HOURS

The hyperthermia normally evoked by AVP following injection into the POA was prevented by the prior administration of $d(CH_2)_5$Tyr(Me)AVP (Figure 21). When 200 ng/0.5 µl/side of the vasopressin antagonist were injected directly into the POA, 60 minutes before AVP (100 ng/0.5 µl/side), the core temperature never increased above baseline values and was significantly reduced for at least three hours following injection ($p < 0.01$, 3 hour fever index). The antagonist alone did not produce a significant change in core temperature of the rats.

4) Discussion

In confirmation of previous reports (Kasting et al., 1980; Meisenberg and Simmons, 1984), the intracerebroventricular administration of AVP elicited a rapid onset, short lasting hypothermia of nearly 1°C. However, since these animals concurrently became immobile it is possible that this decrease in core temperature is of a secondary, non-specific origin. Alternatively, the hypothermia observed after icv vasopressin may be due to an action on a specific brain site. If this is the case, then the six loci tested in this study can be excluded since hypothermia was never observed after direct intracerebral microinjection into these forebrain sites.

The hyperthermic action of vasopressin appears to be site specific. On injection into the POA, AVP elicited an increase in core temperature.

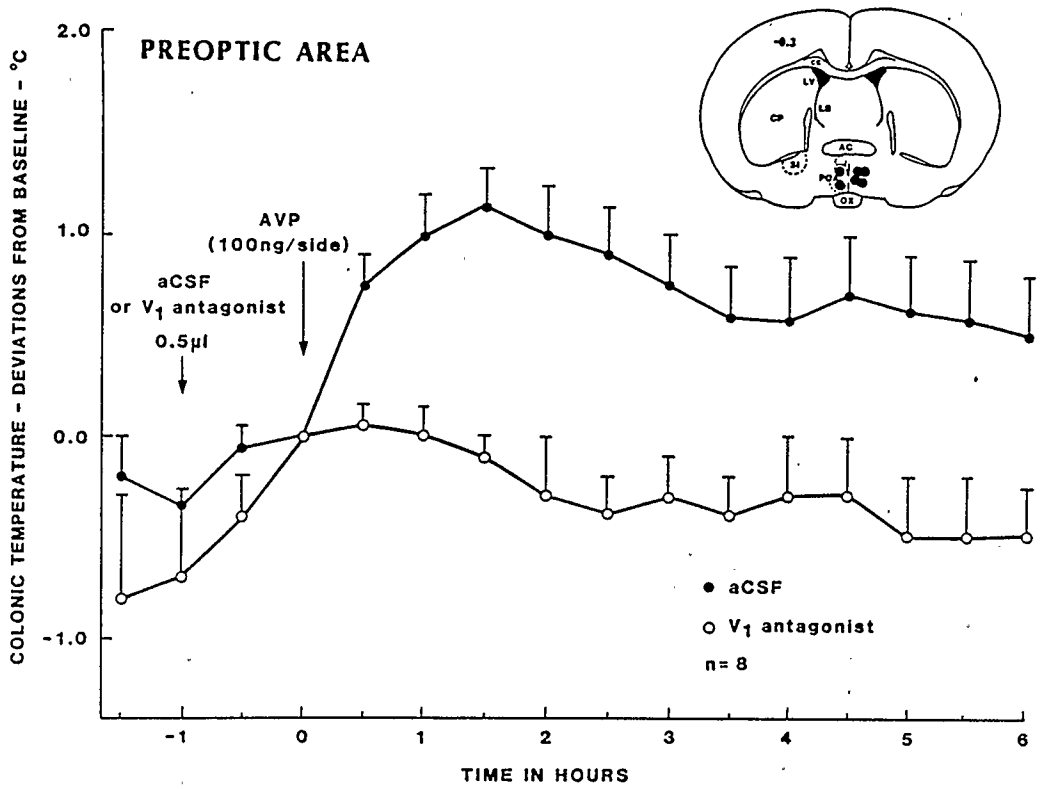



FIGURE 21 Mean temperature responses (± SEM) to direct intracerebral microinjection of aCSF (0.5 µl/side), $V_1$ antagonist $d(CH_2)_5Tyr(Me)AVP$ 200 ng/0.5 µl/side) and AVP (100 ng/0.5 µl/side) into the preoptic area of the rat. The hyperthermia was significantly reduced ($p < 0.01$, 3 hour fever index) in the presence of the AVP antagonist. Abbreviations: AC, anterior commissure; AH, anterior hypothalamus; cc, corpus callosum; CP, caudate putamen; ic, internal capsule; loc, lateral olfactory tract; LS, lateral septum; LV, lateral ventricle; OX, optic chiasm; POA, preoptic area; SI, substantia innominata; 3V, third ventricle.

However, similar injections of AVP into nearby areas of the brain were without effect on the core temperature of the rat. The hyperthermic action of AVP in this area was antagonized by a $V_1$ receptor antagonist (see Kruszynski, et al., 1980). This suggests that the thermogenic effect of AVP may involve specific vasopressin receptors located in the PO/AH.

The findings of Lin and his colleagues (Lin et al., 1983) indicate that a prostaglandin-adrenergic system located in the PO/AH might be involved in the AVP-induced hyperthermia. Our results suggest that, in addition to this system, another important component of the AVP response may rely on the integrity of an AVP receptor population similarly located within the POA. An interaction between prostaglandins and AVP in neuronal systems is supported by several lines of experimental evidence. Administration of prostaglandins of the E series evokes an increase in the release of AVP from organ cultures of the hypothalamus-neurohypophyseal complex of the guinea pig (Ishikawa et al., 1981), or in vivo following intravenous or intracerebroventricular infusion (Vilhardt and Hedquist, 1970; Berl and Schrier, 1973; Andersson and Leksell, 1975; Yamamoto et al., 1976). Whether any prostaglandin-AVP interactions are involved in the hyperthermic effect of intrahypothalamic vasopressin are not clear at present. However, the relationship in thermoregulation between prostaglandins and AVP is strengthened by the demonstration that there is an enhanced urinary excretion of both prostaglandin metabolites and AVP during a fever induced by endotoxin or interleukin-I (Jonasson et al., 1984).

Earlier findings indicate that in addition to its hyperthermic action following intrahypothalamic administration, AVP may act to suppress fever induced by either bacterial pyrogen (Kasting et al., 1979; see earlier) or prostaglandin $E_2$ (see earlier). This antipyretic action is observed only when AVP is administered into the VSA. Indeed, no antipyresis is observed following similar perfusions of the peptide within the PO/AH (Cooper et al., 1979). In the afebrile animal, punctate microinjections of AVP into the VSA exert no thermogenic or thermolytic actions. Thus, vasopressin has a number of thermoregulatory actions when administered intracerebrally that are both anatomically and functionally specific. That is, AVP can be antipyretic when administered into the VSA and hyperthermic when microinjected directly into the PO/AH.

Although previous investigators (e.g., Bernardini et al., 1983; Lipton and Clark, 1986) have found it difficult to resolve how one peptide could subserve the central mechanisms involved in both thermolysis and thermogenesis, the results of the present study may provide a reasonable explanation. A possible reconciliation may lie within a careful neuroanatomical evaluation of the effects of AVP on the thermoregulatory system. Indeed, the present findings coupled with previous investigations detailing the antipyretic action of AVP suggest that this peptide exerts a very different action dependent upon whether it is administered into a lateral ventricle (hypothermia), the POA (hyperthermia) or the VSA (antipyresis).

In conclusion, the intracerebroventricular route of administration may not reveal the entire spectrum of thermoregulatory actions of vasopressin. These findings indicate that it is necessary

to examine specific sites within the brain to more fully delineate the actions of this peptide in maintaining thermal homeostasis. Indeed, dependent upon the specific brain site into which it is injected, AVP may exert a variety of actions affecting widely disparate effector systems.

## B. CONVULSIVE ACTIONS OF VASOPRESSIN

### 1) Introduction

Following infusion of AVP into a lateral cerebral ventricle of the rat, severe motor disturbances including barrel rotations and myotonic/myoclonic convulsions are observed (Kruse et al., 1977; Kasting et al., 1980; Burnard et al., 1983). This behaviour shows a type of sensitization in that an initial injection of AVP predisposes the rats to an increased likelihood of convulsions following a second or subsequent administration of the peptide into the cerebral ventricles (Kasting et al., 1980; Burnard et al., 1983). These convulsive episodes are coincident with an immediate increase in the amplitude and a decrease in the frequency of hippocampal electrical activity followed by cortical spiking (Burnard et al., 1983).

In view of the fact that AVP is released during fever, it has been proposed that central release of AVP during the febrile response may contribute to the pathogenesis of febrile convulsions (Kasting et al., 1981). As yet, the exact neuroanatomical loci in which AVP exerts its convulsive action has not been identified. In this study,

AVP and oxytocin were injected into the VSA (where AVP exerts its antipyretic action) to determine whether this would evoke convulsive behaviour similar to that observed following icv injection of vasopressin.

2) Methods

Male Long-Evans rats were used for the experiments. The surgery, maintenance, injection of peptides into the VSA and histological site verifications are similar to those described previously. Only the occurrence of the most severe motor disturbances were considered: 1) barrel rotations (spinning along the longitudinal axis); 2) myoclonic/myotonic convulsions (muscular rigidity of the forelimbs and hindlimbs and/or clonic spasm or twitching).

3) Results

Convulsive-like behaviour was elicited following bilateral injection of 100 ng of AVP, but not saline, into the VSA (Figure 22). These convulsive episodes followed also the sensitization phenomena described after icv injection of vasopressin. The convulsive behaviours were usually observed within 2 min after injection of AVP. These disturbances were characterized by an initial period of immobility and staring, with some evidence of ataxia. From this state there was a rapid progression to the first of a series of 2-3 episodes of barrel rotations. As the spinning action terminated the rats adopted a posture indicative of a myotonic state. In contrast to AVP,

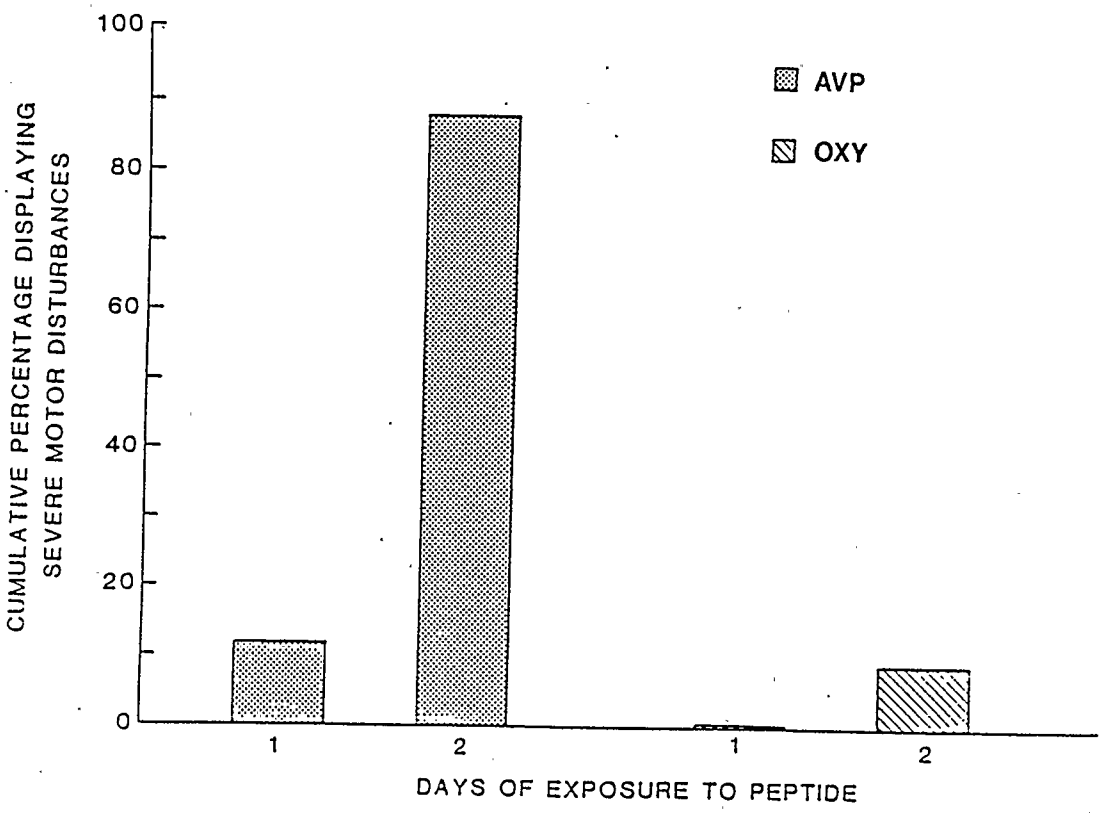



FIGURE 22 Cumulative percentages of rats displaying convulsive behaviour after bilateral injection of AVP (100 ng/side; n = 17) or OXY (100 ng/side; n = 11) into the VSA. Days of exposure to AVP refers to separate injections of peptide carried out at least 1 day apart.

the structurally similar peptide oxytocin (OXY) exhibited little or no convulsive activity when injected into the VSA at the dose tested (100 ng bilaterally).

4) Discussion

Injection of AVP into the VSA evoked convulsive behaviour which was similar to that observed following icv infusion of the peptide. This action was specific to AVP in that the structurally similar neurohypophyseal peptide, oxytocin, had little or no convulsive activity when injected into this area of the brain. Vasopressin, therefore, evokes convulsive activity in the same area of the brain where it suppresses fever. Thus, it is conceivable that an abnormal secretion of AVP in the VSA during fever could initiate events which might contribute to the pathogenesis of febrile convulsion. Other circumstantial evidence linking the antipyretic and convulsive actions of AVP are that both demonstrate site specificity within the VSA (Cooper et al., 1979; see earlier) and both can be blocked by the $V_1$ vasopressin antagonist $d(CH_2)_5$Tyr(Me)AVP (Kasting and Wilkinson, 1986; Naylor et al., 1985; Burnard et al., 1986).

These finding suggest that injection of AVP into the VSA might initiate a response, via projections to the cortex (Fibiger, 1982), which could bring about a cortical involvement similar to that recorded during the convulsive behaviour. However, whether AVP is involved in the pathogenesis of any naturally occurring convulsive disorder remains to be investigated.

# V. EFFECTS OF INTRASEPTAL VASOPRESSIN AND OXYTOCIN ON ENDOTOXIN FEVER

## A. ANTIPYRETIC ACTION OF VASOPRESSIN BUT NOT OXYTOCIN IN THE CAT

### 1) Introduction

These experiments were carried out in the cat to determine whether AVP exerts a dose-related antipyretic action when perfused in ventral septal sites during a fever evoked by bacterial pyrogen administered intracerebroventricularly. In addition, oxytocin, a structurally similar peptide to AVP was tested for its antipyretic activity by perfusing it in the same sites where AVP suppressed fever since some of the central actions of vasopressin have been attributed to an action on an oxytocin receptor (Mühlethaler et al., 1983).

### 2) Methods

Mongrel cats of either sex (6) were housed individually at an ambient temperature of 21 ± 2°C on a 12 hour light/dark cycle. Under pentobarbital anaesthesia (27.5 mg/kg intravenously) an array of 4-6 guide cannulae was implanted stereotaxically (Jasper and Ajmone-Marsan, 1954) according to procedures described earlier. The guide cannulae were positioned bilaterally above the VSA and anterior hypothalamic/preoptic area and unilaterally above a lateral cerebral ventricle. Following a 14 day recovery period, a series of push-pull perfusions were undertaken in each cat (see earlier). AVP (Bachem)

6.5 ng or 13.0 ng/µl, oxytocin (OXY; Bachem) 13.0 ng/µl, or aCSF (Schwartzkroin, 1975) were perfused at a rate of 16.0 µl/min into sites within the VSA. The stock solution of AVP was initially diluted in 0.025 M acetic acid. Thereafter, each aliquot of AVP could be diluted to the appropriate concentration with sterile aCSF. Oxytocin was dissolved similarly in aCSF. One hour after commencement of perfusion, Salmonella typhosa (Difco laboratories; 100 ng/50 µl) was infused via gravity flow into a lateral cerebral ventricle (icv). After this injection, the perfusion was continued for an additional 3 hours. Similar push-pull perfusions carried out in the absence of pyrogen were undertaken to determine the thermoregulatory effects of these neuropeptides in the afebrile animal.

To measure core temperature in the conscious and unrestrained cat a YSI 701 thermistor probe was inserted 10 cm beyond the anus and held in place by surgical tape wrapped around the tail. Colonic temperature was monitored and recorded simultaneously on a Digitec datalogger. The baseline temperature of each cat, recorded at 5-15 min intervals, was obtained for at least 90 min prior to the commencement of an experiment and continued thereafter until completion of the experiment.

To identify sites at which perfusions were carried out, each cat was anaesthetized deeply with sodium pentobarbital. Saline, followed by formalin was perfused through the brain. The brain was then sectioned on a freezing microtome at 75 µm thick slices and sections were mounted on glass slides and stained with neutral red. Locations of the perfusion sites were then identified using light microscopy.

Data were analyzed according to Student's t distribution for paired subjects. A fever index was computed for each response, that is, the area under the temperature-time curve was expressed as degrees centigrade hours for 4 hr following injection of the pyrogen.

3) Results

Figure 23 illustrates the time course of the fever evoked by icv injection of Salmonella typhosa expressed as the mean response for 4 cats. Perfusion of the carrier vehicle alone, within the VSA, did not alter the normal development of the fever. However, when AVP (13.0 ng/μl) was perfused similarly, there was a consistent suppression of the fever during the period of perfusion with the peptide (Figure 23).

In order to determine the specificity of action of AVP in suppressing pyrogen-induced fever the structurally similar neurohypophyseal peptide, oxytocin, was tested for its antipyretic effects within the brain. Figure 23 demonstrates that perfusion of oxytocin, at the highest concentration tested, within the AVP sensitive antipyretic sites in the VSA did not alter the development of the fever produced by icv Salmonella typhosa.

One estimation of the magnitude of the febrile response, the fever index (expressed in °C.h for 4 hours), indicates that the fever was significantly altered in the presence of AVP. Perfusion of the lower dose of AVP (6.5 ng/μl) often delayed the onset of fever (Figure 24) and reduced the absolute magnitude of the febrile response (Figure 25). An analysis of the fever index demonstrated a significant reduction in the febrile response ($p < 0.05$) using this concentration

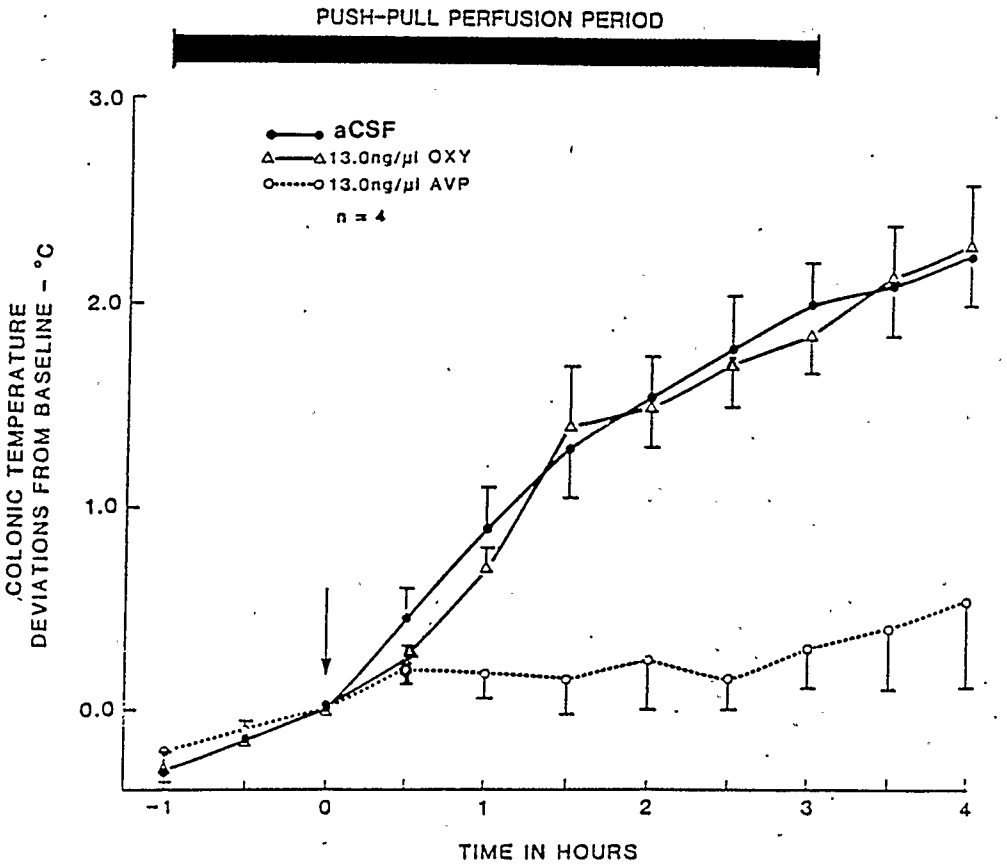



FIGURE 23 Mean colonic temperature responses (± SEM) of cats following an injection of 100 ng/50 µl of Salmonella typhosa into the lateral cerebral ventricle at time zero (arrow). Perfusion of AVP within the VSA completely suppressed the febrile response whilst oxytocin was without effect on the hyperthermia evoked by pyrogen.

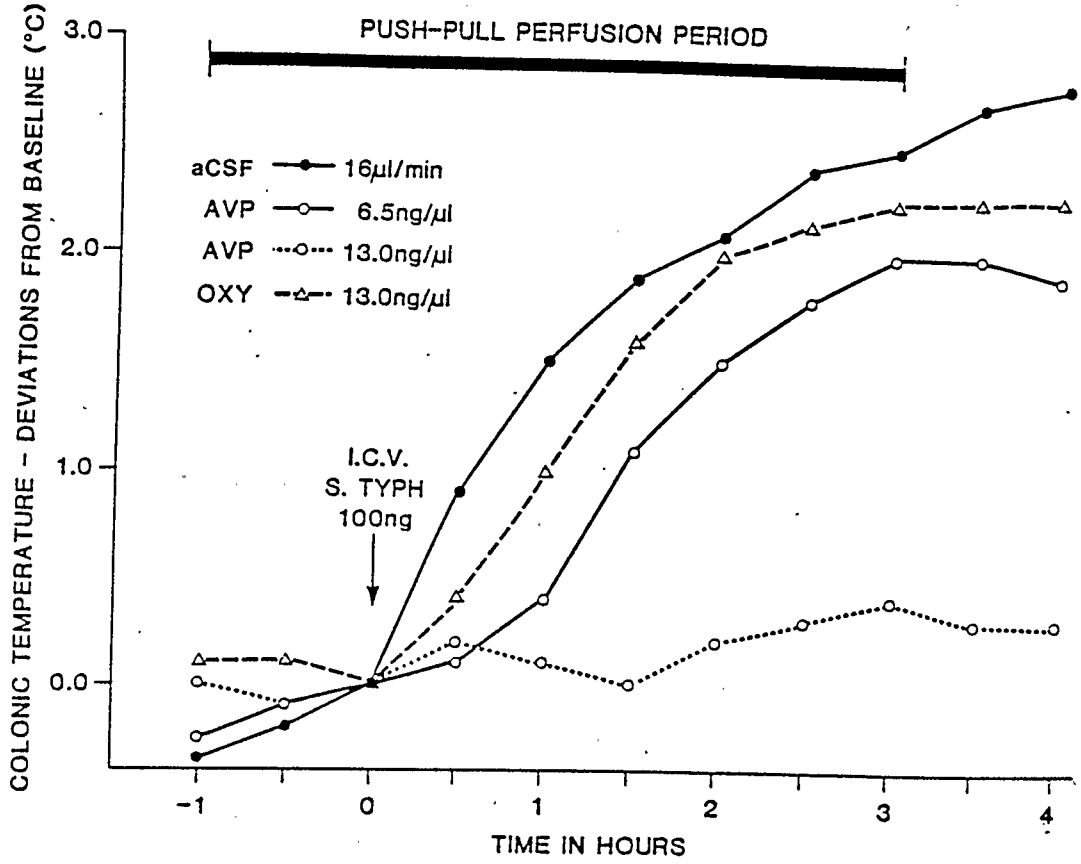



FIGURE 24 Colonic temperature in deviations from baseline (°C) in a cat demonstrating the effects of perfusion of AVP (2 doses) within the VSA on fever evoked by icv endotoxin. In this case, 6.5 ng/µl AVP suppressed initially the rise in core temperature.

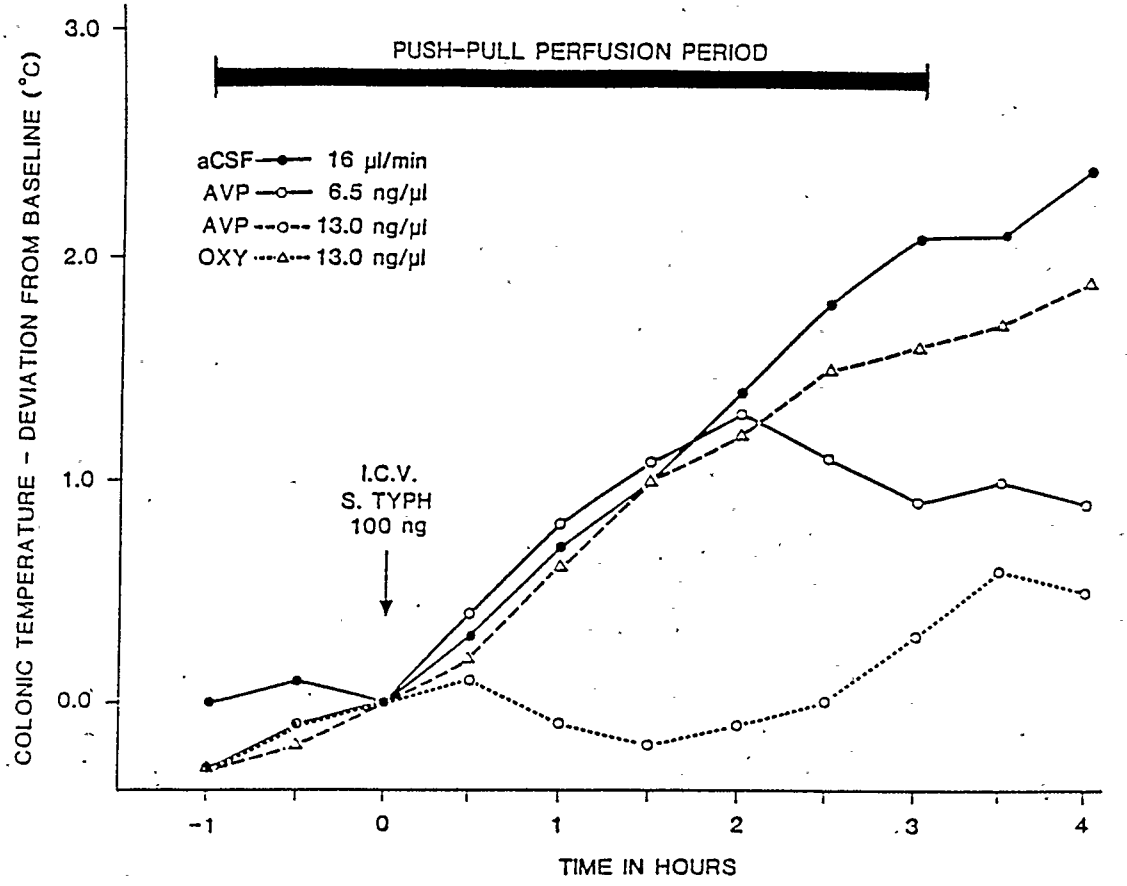



FIGURE 25 Colonic temperature in deviations from baseline (°C) in a cat demonstrating the effects of perfusion of AVP (2 doses) within the VSA on fever evoked by icv endotoxin. In this case, 6.5 ng/µl AVP reduced the absolute magnitude of the rise in core temperature.

of AVP as compared to administration of aCSF (Figure 26). When the concentration of AVP was increased to 13.0 ng/µl, the fever index was again reduced significantly ($p < 0.01$) (Figure 26). In contrast, perfusion of oxytocin was ineffective in altering the febrile response evoked by bacterial pyrogen. Core temperature responses during bilateral perfusion of the VSA with 13.0 ng/µl of AVP and OXY, in the absence of pyrogen, were also monitored. This concentration of peptide had no effect on the core temperature of the afebrile cat when perfused within these brain sites (Figure 27).

The location of the push-pull perfusion sites at which the fever was attenuated by AVP are depicted in the schematic histological sections presented in Figure 28. Sites at which perfusion of this peptide was ineffective in producing antipyresis are likewise presented in this figure. Sites at which AVP was effective in producing antipyresis were located predominantly within the more ventral aspects of the septal area.

4) Discussion

Intracerebroventricular administration of Salmonella typhosa evoked a prominent febrile response in the cat which was not affected by the concomitant bilateral perfusion of the ventral septal area with aCSF. This increase in core temperature was coincident with a decreased rate of respiration, piloerection, huddled posture and whole body shivering. When AVP was present in the perfusion medium bathing the VSA, the fever evoked by the pyrogen was reduced significantly. The higher dose of AVP consistently suppressed the fever throughout

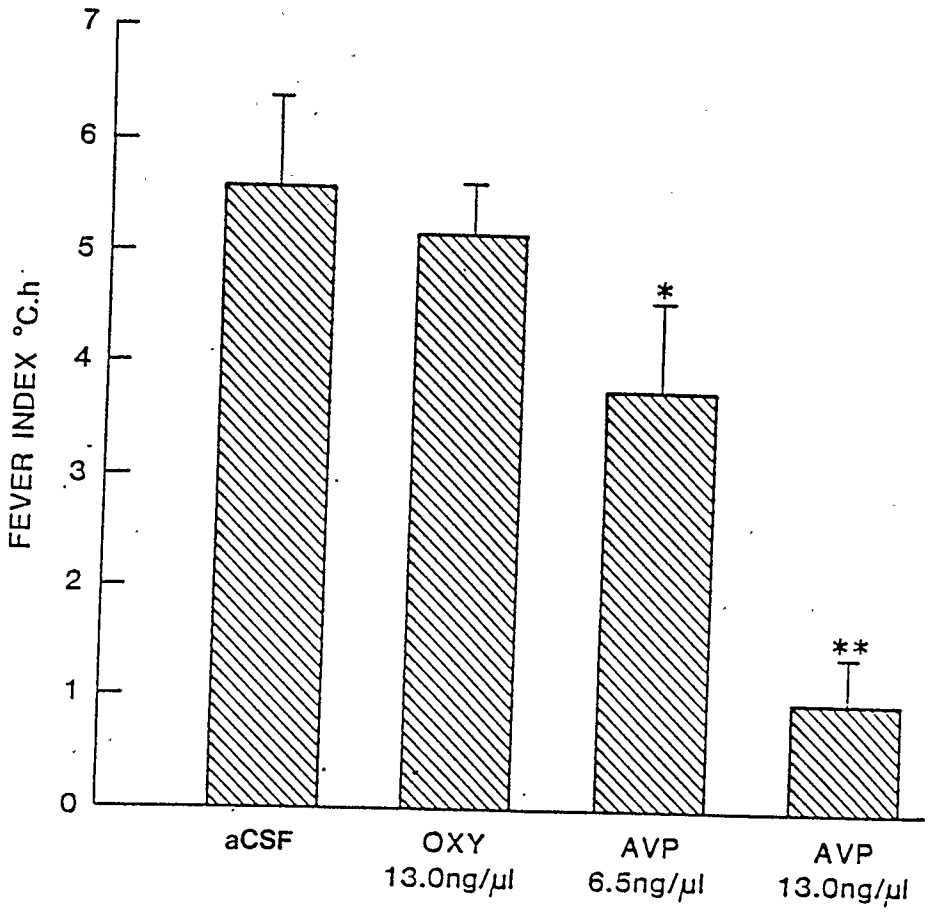



FIGURE 26 Fever indices (± SEM; calculated over 4 hours) obtained following intracerebroventricular (icv) administration of Salmonella typhosa into the conscious and unrestrained cat. (N.S., not significantly different from aCSF; * $p < 0.05$; ** $p < 0.01$ in comparison to control perfusions of aCSF.)

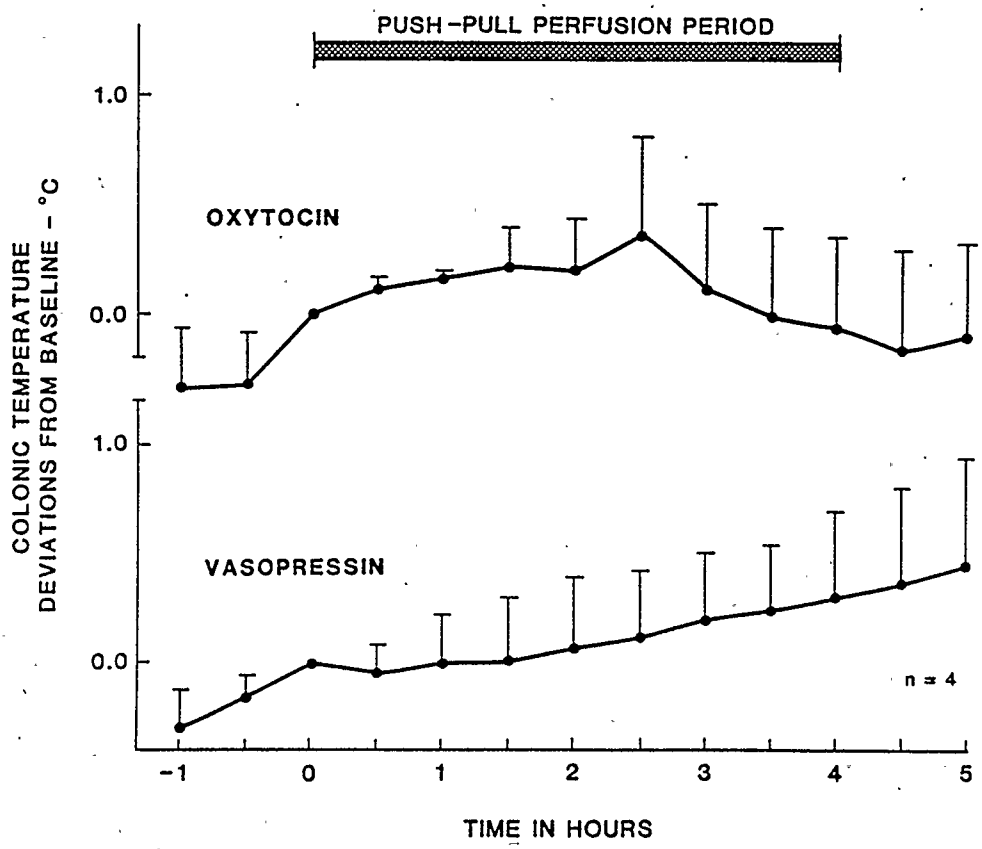



FIGURE 27 Mean colonic temperature responses (± SEM) of cats following perfusion of oxytocin (upper) or vasospressin (lower) within the VSA of afebrile animals.

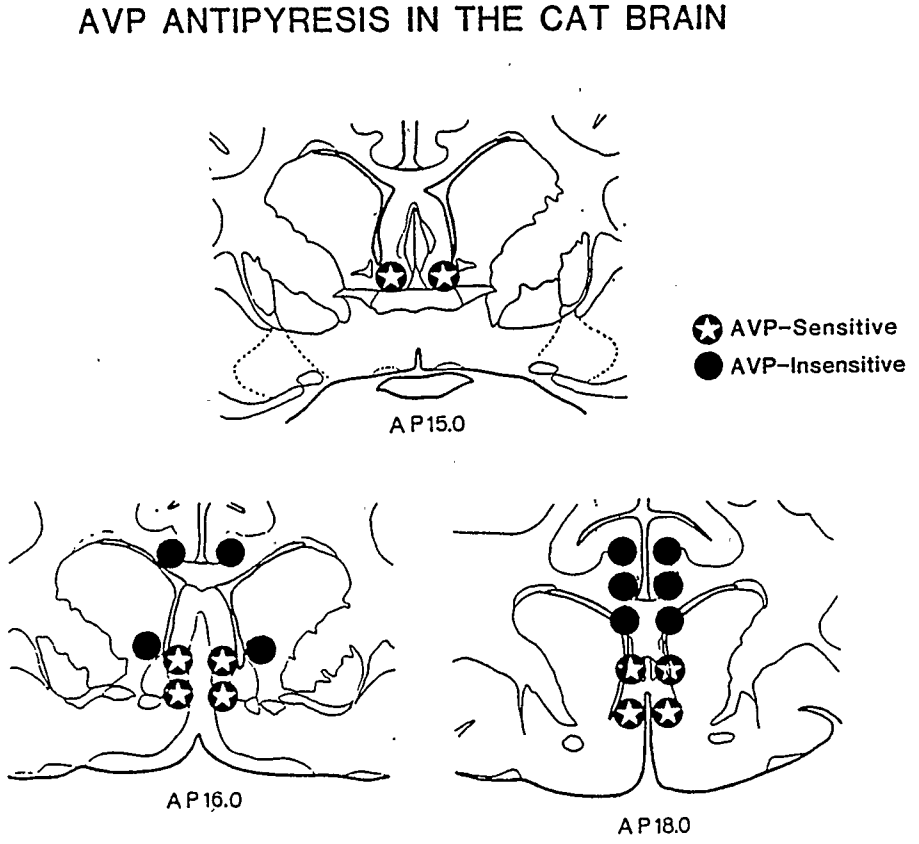



FIGURE 28 Schematic histological sections of the cat brain indicating sites at which vasopressin either suppressed (circles with stars) or had no effect (closed circles) on the febrile response to Salmonella typhosa administered intracerebroventricularly.

the period of administration and, in some cases, an increase in core temperature was not observed even upon termination of the perfusion. Perfusion of the lower dose of AVP delayed the onset of fever and in some cases reduced the absolute magnitude. In those cases in which the fever was suppressed by administration of AVP, the animals did not adopt postures indicative of a febrile state and did not manifest changes in physiological mechanisms used to conserve and/or produce body heat.

Administration of the structurally similar neurohypophyseal peptide, oxytocin, into AVP-sensitive antipyretic sites in the VSA was without effect on the febrile response. In another study employing oxytocin (Kovacs and De Wied, 1983) the icv administration of this peptide was similarly found to be ineffective in reducing fever. This suggests that the ability of AVP to suppress pyrogen fever is specific to this peptide. In support of this, other neuropeptides including somatostatin, substance P and angiotension II are ineffective in attenuating fever (Kasting, 1980). Further, it suggests that unlike other central effects of vasopressin (Mühlethaler et al., 1982, 1983), the antipyretic action of AVP does not involve an interaction with a central oxytocin receptor. It is unlikely that the reduction in fever observed with AVP was due to a non-specific reduction in core temperature since perfusion of the peptide alone within active sites in the VSA had no effect on the normal body temperature of afebrile cats. Similarly, oxytocin at the highest concentration tested did not alter the core temperature of these animals.

The icv route of pyrogen administration was chosen because tolerance to the febrile effects of endotoxin are not observed under such conditions. Tachyphylaxis to administration of bacterial pyrogen does occur, however, when administered intravenously (Sheth and Borison, 1960). The push-pull perfusion technique was utilized to administer AVP into brain tissue because it permits a constant amount of peptide to be delivered over a long period of time - characteristics that cannot be reproduced by a single bolus microinjection of neuroactive substances. In addition, these features make push-pull perfusion particularly useful for investigations into physiological events, like fever, which occur over a number of hours.

Sites in the brain which appear to mediate the antipyretic action of AVP are located in a region ventral to the medial and lateral septum, and which are bounded by the diagonal bands of Broca and the anterior commissure. In addition, the region of greatest sensitivity to AVP appears to course caudally in the brain and may extend to an area just dorsal to the anterior commissure into the posterior septal nucleus. In the rat, these active sites are richly innervated by immunoreactive vasopressin containing fibres (Buijs et al., 1978; De Vries et al., 1985; Swanson, 1977). In addition, specific receptor binding sites (Baskin et al., 1983) for AVP are located within the VSA. The sites where perfusion of AVP did not alter the characteristics of the febrile response were located primarily in a dorsal-lateral direction from the AVP-sensitive areas. Interestingly, the sites in the forebrain where AVP reduces fever are similar to brain areas where vasopressin levels rise (i.e. septum) in the pregnant guinea-pig during the period of diminished febrile

response observed at term (Merker et al., 1980). This suggests that the two events may be related. However, more work is required before this can be stated with certainty.

In confirmation of recent reports regarding the antipyretic action of neuropeptides, the icv administration of AVP during fever did not result in any consistent reduction in the febrile response in the cat (personal observation). This absence of antipyretic action following ventricular injection of AVP has been reported previously for the rabbit (Bernardini et al., 1983; see earlier) and monkey (Lee et al., 1985) and may reflect an inability of the peptide to reach important antipyretic sites within the VSA.

This study provides further evidence that AVP may act as an endogenous antipyretic within the mammalian brain, thus supporting the idea that this neuropeptide is involved in the central mechanisms that regulate core temperature. Perfusion of vasopressin within sites located in the VSA suppressed fever evoked by pyrogen in a dose-dependent manner. This antipyretic action was specific to AVP in that oxytocin was without effect on the febrile response. Furthermore, these data extend the previously documented antipyretic actions of vasopressin to another species, the cat. Utilizing this animal with its well defined neuroanatomical structures, it has been possible to provide a more precise delineation of those areas of the forebrain in which AVP effects an antipyretic action.

## B. ANTIPYRETIC ACTION OF VASOPRESSIN BUT NOT OXYTOCIN IN THE GUINEA-PIG

### 1) Introduction

The guinea-pig is one species where there is direct evidence for the involvement of vasopressin in the suppression of fever observed at term. Specifically, around parturition, levels of AVP (determined immunocytochemically) increased in neurons of the paraventricular nucleus and within nerve terminals of the amygdala and septum when fever was absent (Merker et al., 1980). The area of the septum in which vasopressinergic neurons increased is similar to where, in other species, exogenous administration of AVP can suppress fever. These experiments were undertaken in the non-pregnant guinea-pig to determine whether administration of vasopressin within this area of the brain (VSA) could suppress endotoxin fever. In addition, the specificity of this action was examined using the structurally similar peptide oxytocin.

### 2) Methods

Male albino guinea-pigs were maintained in a light/dark cycle of 12 hours. Food and water were available ad libitum.

Bilateral stainless steel guide cannulae (20 ga) were implanted stereotaxically above the septum, under pentobarbital anesthesia (37 mg/kg/ i.p.), according to the stereotaxic coordinates of Tindal (1965). An additional guide cannula was implanted above a lateral

cerebral ventricle and all cannulae were affixed to the calvaria with dental cement and stainless steel screws. Body temperature measurement, push-pull perfusion of AVP (Bachem) and Oxytocin (Bachem), icv injection of SAE and perfusion site verification were similar to those described previously.

3) Results

Figure 29 illustrates the time-course of the febrile response in two individual guinea-pigs. Perfusion of the carrier vehicle alone within the VSA (see histological insets) did not alter the normal development of fever. However, when AVP was perfused similarly, the normal rise in body temperature did not occur. There was an elevation in body temperature towards the end of the perfusion period. In contrast, perfusion of oxytocin within these AVP-sensitive antipyretic sites in the VSA did not result in any fever suppression (Figure 29). Figure 30 demonstrates the site specificity of the AVP-induced antipyresis. In these two guinea-pigs, perfusion of AVP within more dorsal components of the septum resulted in no suppression of fever.

4) Discussion

These data indicate that AVP can suppress endotoxin fever in the non-pregnant guinea-pig. This action of AVP is site specific and peptide specific since perfusion of AVP outside the VSA, or perfusion of oxytocin in the VSA, did not result in any fever suppression. Furthermore, the area of the guinea-pig brain where AVP suppressed

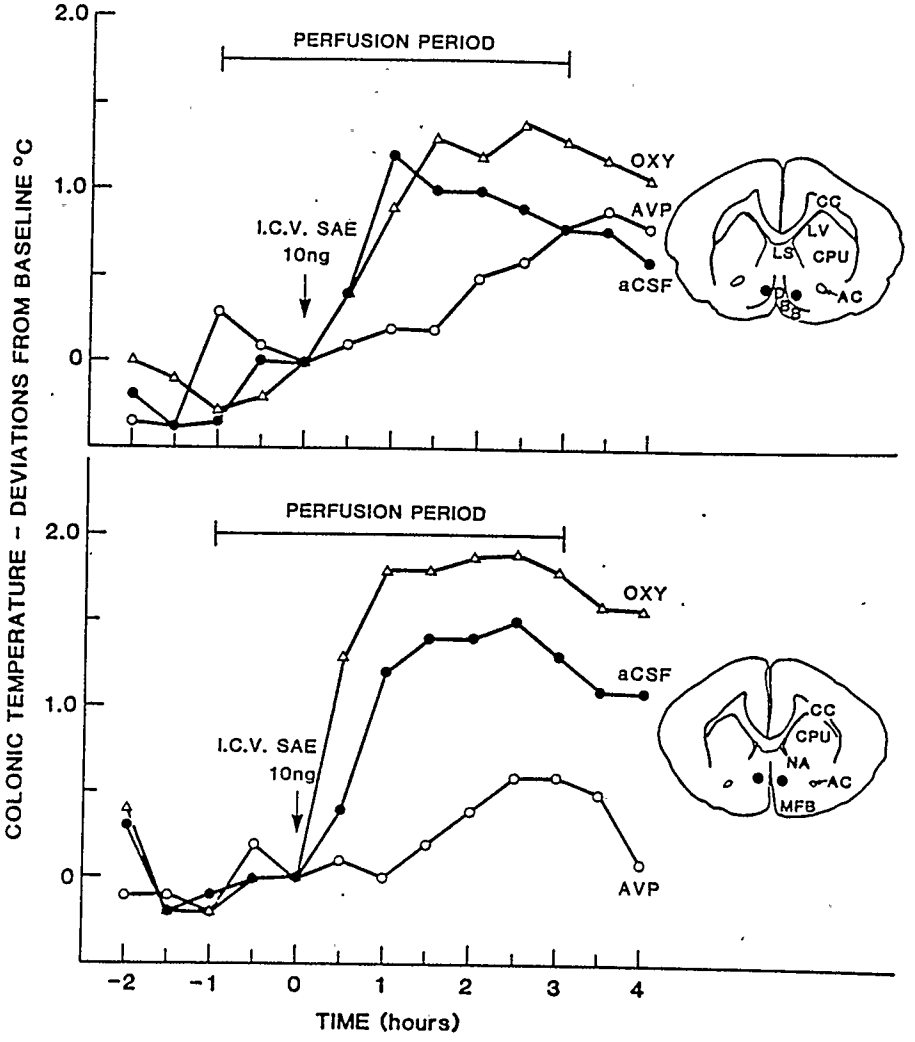



FIGURE 29 Colonic temperature in deviations from baseline (°C) in two conscious guinea-pigs demonstrating where perfusion of AVP suppressed pyrogen fever (see insets). Arginine-vasopressin, (AVP, open circles), oxytocin (OXY, open triangles) (both at 6.5 ng/µl) or artificial cerebrospinal fluid (aCSF) were perfused (30.0 µl/min) into the ventral septal area for 1.0 hr prior to, and 3.0 hr. following an intracerebroventricular (icv) injection of Salmonella abortus equi (SAE, 10.0 ng/10.0 µl).
Abbreviations: AC, anterior commissure; cc, corpus callosum; CPU, caudate putamen; DBB, diagonal band of Broca; LS, lateral septum; LV, lateral ventricle; NA , nucleus accumbens; OC, optic chiasm.

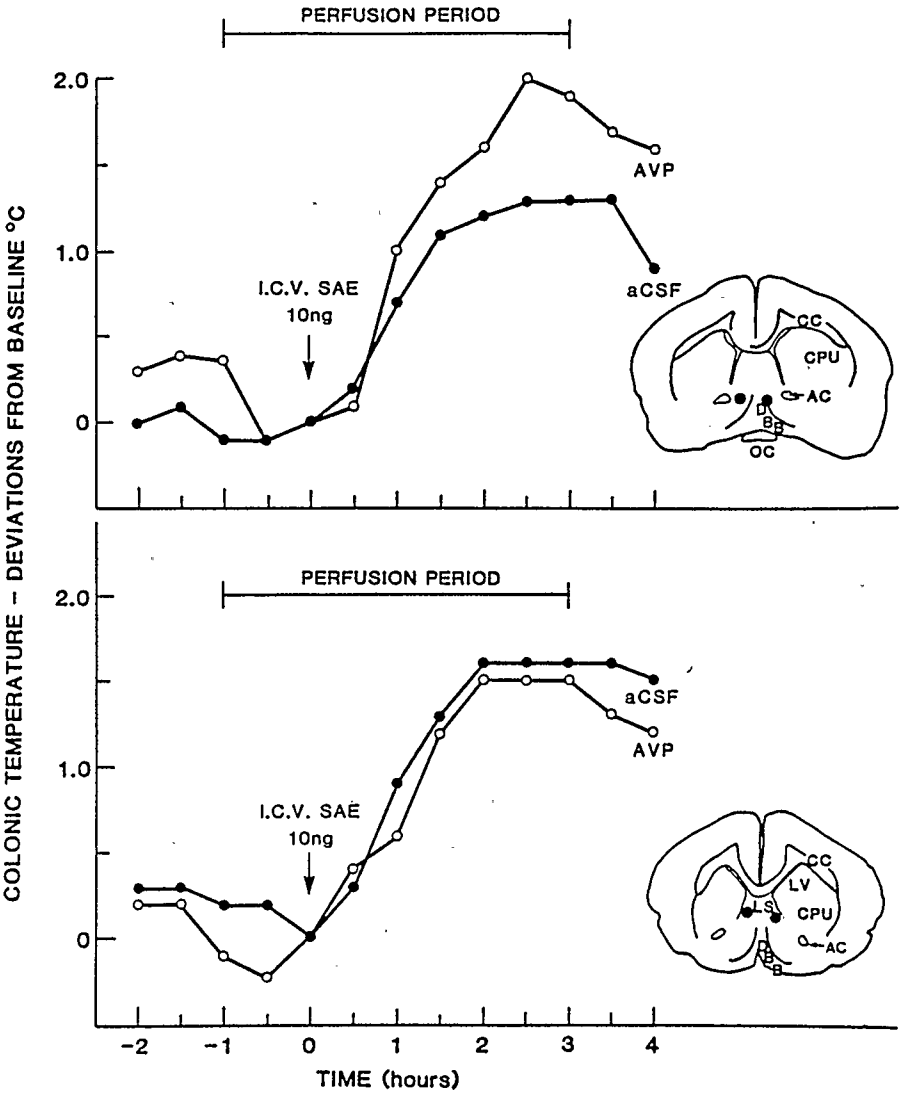



FIGURE 30 Colonic temperature in deviations from baseline (°C) in two conscious guinea-pigs demonstrating where perfusion of AVP did not suppress pyrogen fever (see insets). Arginine vasopressin (AVP-open circles; 6.5 ng/µl) or aCSF (closed circles) were perfused (30.0 µl/min) into sites outside the ventral septal area for 1.0 hr prior to, and 3.0 hr following an icv injection of SAE (10.0 ng/10.0µl).
Abbreviations: see legend for Figure 29.

fever is similar to that where immunoreactive AVP is altered during the absence of fever observed at term (Merker et al., 1980). This suggests that the two events may be related. However, more work and evidence is required before this can be stated with certainty.

# VI. CHARACTERISTICS OF THE CENTRAL RECEPTOR MEDIATING THE ANTIPYRETIC ACTION OF VASOPRESSIN

## A. EFFECT OF VASOPRESSIN ANALOGUES ON VASOPRESSIN-INDUCED ANTIPYRESIS

### 1) Introduction

The nature of the central receptor mediating the antipyretic action of AVP is not known at this time. In the periphery, two subtypes of vasopressin receptor have been well characterized ($V_1$-vasopressor and $V_2$-antidiuretic). This classification is based on differing ligand selectivities and also on the second messenger involved (Mitchell et al., 1979). In the CNS, there is evidence that the blood pressure (Pittman and Franklin, 1985) and convulsive (Naylor et al., 1985; Burnard et al., 1986) actions of intracerebral vasopressin are mediated via a receptor which resembles the peripheral $V_1$ (vasopressor) subtype. In addition, a depolarizing effect of AVP on supraoptic neurons in the hypothalamic slice has been attributed to an action on a central $V_2$ receptor (Abe et al., 1983).

Experiments were carried out in the rat to determine whether in addition to reducing the febrile response to endotoxin and prostaglandin $E_2$, AVP could suppress the fever evoked by interleukin-1 (endogenous pyrogen), the primary mediator of the host defence response (Dinarello, 1984a). Further, the experiments were designed to determine whether the central receptor mediating the antipyretic action of vasopressin resembled the $V_1$ or $V_2$ subtype of peripheral

vasopressin receptor. This was achieved using a relatively specific antagonist of the $V_1$ subtype of vasopressin receptor [$d(CH_2)_5$Tyr(Me)AVP] (Kruszynski et al., 1980) and a selective agonist for the $V_2$ subtype (1-desamino-8-D-arginine vasopressin; DDAVP) (Sawyer et al., 1974).

2) Methods

Male Long-Evans rats (280-320g) were used for the experiments and were maintained at an environmental temperature of 25.0 ± 1°C. Animals had ad libitum access to water and standard laboratory rat chow. Under barbiturate-induced anesthesia, a guide cannula (20-gauge TW) was implanted stereotaxically (Paxinos and Watson, 1982) so that the tip rested above a lateral cerebral ventricle. Animals recovered from surgery for a period of 7 days.

Prior to experimentation each rat was placed individually in a plastic cage, without restraint, for at least 120 min before infusion of the peptide or control solution (sterile physiological saline). Stock solutions of AVP(Bachem), DDAVP (Ferring) and $d(CH_2)_5$Tyr(Me)AVP (supplied by Dr. M. Manning) were maintained at 4°C. Immediately prior to the injection, peptides were diluted to the appropriate concentration with sterile saline. A highly purified form of interleukin-1 was supplied by Dr. C.A. Dinarello. For icv injections, a needle was lowered down the guide cannula to the appropriate depth and the peptide IL-1 or control solution was infused, by gravity flow, into conscious rats in a volume of 5.0 µl. The order of injection was randomized prior to experimentation. For 120 min prior to and 240 min

following the injection of IL-1, the colonic temperature of each animal was monitored continuously using a thermistor probe (YSI701) inserted 6 cm beyond the anus and taped to the tail. The core temperature of each rat was recorded at 5-15 min intervals on a digital datalogger (Digitec).

Upon completion of the experiments, 5.0 µl of blue dye were injected through the cannula and the ventricular spaces were examined to verify that the injections had been made into a lateral cerebral ventricle. The results were assessed statistically using an analysis of variance followed by the Scheffe post-hoc test.

3) Results

Intracerebroventricular administration of purified IL-1 (5.0 µl) evoked a rise in core temperature which was not altered by the subsequent injection, 15 min later, of saline (5.0 µl) into a lateral ventricle (Figure 31). Similarly, when the $V_2$ receptor agonist DDAVP (5.0 pmoles/5.0 µl) was injected icv 15 min following IL-1, the fever in response to IL-1 followed the same time course as the control response (with IL-1 + saline). In contrast, when AVP (5.0 pmoles/5.0 µl) was injected into the brain following an icv injection of IL-1, the time-course of the fever was altered. Instead of the core temperature rising to approximately 1°C 30 min after icv IL-1 (as with the saline and DDAVP treated group), the temperature of the rats that received AVP did not change from pre-AVP injection levels. The antipyretic action of AVP was significantly different from the saline

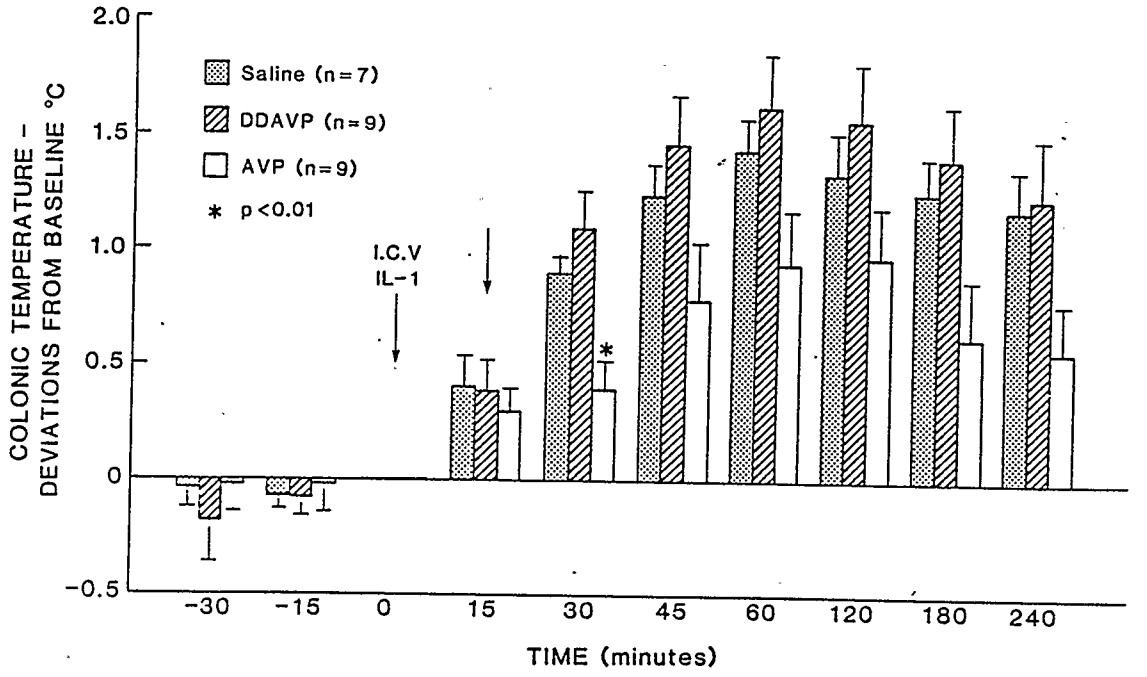



FIGURE 31

Mean temperature responses (± SEM) of rats following infusion of purified interleukin-1 into a lateral ventricle at time zero. Saline (5.0 µl), DDAVP (5.0 pmoles) or AVP (5.0 pmoles) were injected into a lateral ventricle 15 minutes following the injection of interleukin-1 (arrow). Arginine-vasopressin, but not DDAVP suppressed significantly the febrile response. (* $p < 0.01$)

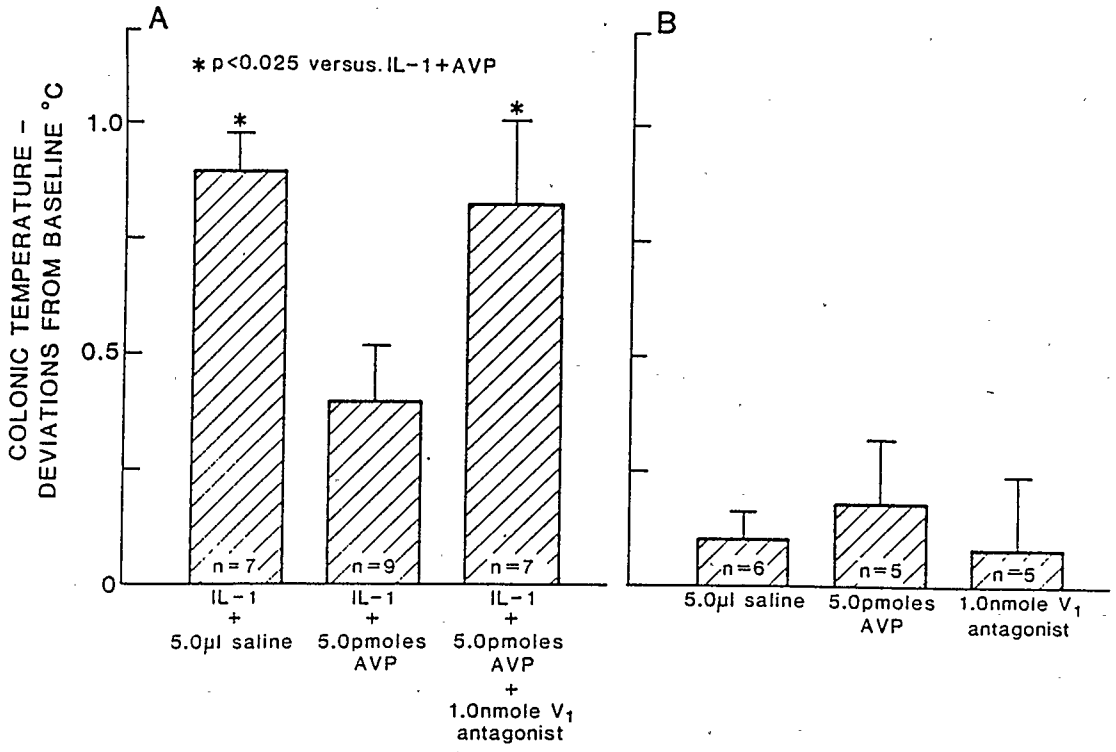



FIGURE 32 A) Mean fever responses (± SEM) of rats following infusion of purified interleukin-1 into a lateral ventricle. The antipyretic effect of AVP was prevented by pretreatment (30 min before the interleukin-1) with the $V_1$ receptor antagonist $d(CH_2)_5Tyr(Me)AVP$, similarly injected into a lateral cerebral ventricle.
B) Mean temperature responses (± SEM) of rats following infusion of saline, AVP and $d(CH_2)_5Tyr(Me)AVP$ into a lateral ventricle, without any injection of interleukin-1. (* $p < 0.025$).

and DDAVP treated group (Fig 31). Throughout the period of recording (240 min), the febrile response was significantly greater in the DDAVP treated rats as compared to those that received AVP.

In a second series of experiments, the ability of a relatively selective $V_1$ antagonist, $d(CH_2)_5Tyr(Me)AVP$ (Kruszynski et al., 1980) to antagonize the antipyretic action of AVP was investigated. Pretreatment of the rats with the $V_1$ antagonist (1.0 nmole/5.0 µl) blocked completely the fever reducing properties of AVP ($p < 0.025$; Figure 32A). Also shown in Fig. 32 (Fig 32B), the intracerebroventricular infusion of saline, AVP or the $V_1$ antagonist was without effect on the core temperature of the rats at the doses tested. Similarly, DDAVP alone did not evoke any changes in core temperature (data not shown).

4) Discussion

Intracerebroventricular administration of highly purified IL-1, derived from human monocytes, evoked a short latency rise in core temperature which was sustained for up to 4 hours. Injection of AVP into the brain, 15 minutes after IL-1, prevented the normal development of the febrile response. Therefore, as well as being antipyretic against endotoxin and prostaglandin $E_2$ fever, AVP also can reduce the rise in body temperature in response to the central injection of IL-1. Since infusion of AVP alone did not affect resting body temperature it is unlikely that the reduction in IL-1 fever is due to a non-specific decrease in normal core temperature. The duration of the antipyretic action of AVP was approximately 15-20 min

following a single injection of 5.0 pmoles of the peptide into the brain. This is not surprising in view of the fact that intracerebroventricularly administered vasopressin has been reported to have a half-life in cerebrospinal fluid of only a few minutes (Meisenberg and Simmons, 1984b).

In the periphery, an action of AVP on $V_1$ and $V_2$ receptors is believed to be responsible for the vasopressor and antidiuretic effects respectively of the peptide. Currently, it is not known whether the central receptor mediating the antipyretic action of AVP resembles the peripheral $V_1$ or $V_2$ subtype or whether there is another class of central receptor which is neither $V_1$ nor $V_2$ in nature. However, direct evidence exists to support the presence of vasopressin receptors in the CNS, including the VSA (Baskin et al., 1983) where AVP is antipyretic (see earlier. The results from this study utilizing the selective $V_2$ agonist, DDAVP, suggest that the receptors mediating the antipyretic action of AVP are unlikely to resemble the peripheral $V_2$ subtype since DDAVP is devoid of antipyretic activity against IL-1 induced fevers. The use of the relatively selective $V_1$ receptor antagonist, $d(CH_2)_5Tyr(Me)AVP$, implies that the central receptors responsible for the antipyretic effect of AVP may resemble those at which AVP elicits its pressor effects, i.e. $V_1$. This is because the $V_1$ antagonist can antagonize the antipyretic action of exogenously administered AVP but has little or no effect on body temperature when injected alone, suggesting that a non-specific action of the antagonist is unlikely to be responsible (Porter and Brody, 1986).

Since $d(CH_2)_5$Tyr(Me)AVP is a selective vasopressor antagonist (Kruszynski et al., 1980), there is a possibility that the observed antipyretic effects of AVP are due to local changes in cerebral blood flow. However, since another potent vasoconstrictor, angiotensin II, is not antipyretic (Kasting, 1980), this is unlikely. In addition, although $d(CH_2)_5$Tyr(Me)AVP also is a weak oxytocin antagonist (Kruszynski et al., 1980), it does not appear that its actions in this study are mediated via an action on oxytocin receptors since oxytocin does not show any antipyretic activity against pyrogen fevers (Kovacs and De Wied, 1983; see earlier).

The ratio of antagonist:agonist concentration required to block the antipyretic effect of AVP was 200:1, with lower concentrations of the antagonist being ineffective in blocking the vasopressin-induced antipyresis (personal observation). Thus, the $V_1$ receptor antagonist, although able to prevent the antipyretic action of vasopressin, is not very potent in its action. The prevention of AVP-induced antipyresis by a $V_1$ antagonist has also been reported by Kasting and Wilkinson (1986).

In conclusion, these data indicate that AVP can suppress the fever evoked by icv IL-1. In addition, this antipyretic action of vasopressin (like many other central effects of the peptide; Albers et al., 1986; Burnard et al., 1986; Naylor et al., 1985; Pittman and Franklin, 1985) may be mediated by central receptors which resemble the $V_1$ subtype of peripheral vasopressin receptor. The results obtained with the $V_2$ receptor agonist suggest that the receptor is unlikely to resemble the $V_2$ subtype. However, these studies do not exclude the possibility that the antipyretic action of AVP may be

mediated via an interaction with a central receptor which is neither classically $V_1$ or $V_2$. Future studies will utilize vasopressin antagonists to investigate further the role of endogenous vasopressin in the natural suppression of fever.

# VII. VASOPRESSIN AND ENDOGENOUS ANTIPYRESIS IN THE RAT

## A. EVIDENCE SUPPORTING A ROLE FOR ENDOGENOUS VASOPRESSIN IN FEVER SUPPRESSION

### 1) Introduction

Evidence in support of a role for AVP as an antipyretic within the VSA has been obtained in a number of species. Evidence to support the hypothesis that vasopressin might function in this area of the brain under physiological conditions, as an endogenous antipyretic agent (Veale et al., 1981), has been demonstrated in the sheep. During the course of fever the release of vasopressin is altered within the same brain sites where perfusion of vasopressin suppresses fever (Cooper et al., 1979). Therefore, when returned push-pull perfusates, derived from the VSA, were assayed for AVP the amount of peptide present correlated negatively with changes in body temperature. As body temperature rose, smaller amounts of AVP were released into the extracellular fluid and, as the temperature fell, the amount of AVP secretion rose. A similar negative correlation between AVP release and febrile response has been reported for the rabbit (Ruwe et al., 1985).

Currently, the nature of the central receptor(s) mediating the actions of vasopressin on the CNS are not fully understood. The experiments reported in this paper were carried out to determine the role of endogenous vasopressin in fever suppression and to characterize further the nature of the central receptor mediating the

antipyretic action of vasopressin in the rat using vasopressin antagonists. Therefore, two vasopressin antagonists, relatively specific towards the $V_1$ and $V_2$ subtype of peripheral vasopressin receptor respectively (Kruszynski et al., 1980, Manning et al., 1982), were injected into the area of the brain (VSA) where exogenous AVP reduces fever.

2) Methods

Male Long-Evans rats (280-350 g) were used for the experiments. Animals were maintained on a 12 h light/dark cycle at an environmental temperature of 25 ± 1°C. Each animal had ad libitum access to water and standard laboratory rat chow.

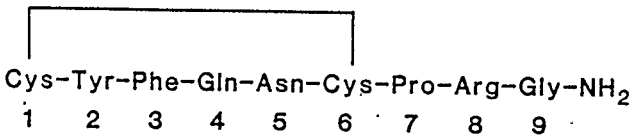
Under anaesthesia (ketamine, 90 mg/kg; xylazine, 1.1 mg/kg, i.m.) bilateral 20 ga (thin wall) stainless steel guide cannulae were implanted stereotaxically (Paxinos and Watson, 1982) so that the tips remained 5 mm above the intended site of injection in the VSA. In addition, a single guide cannula was implanted above a lateral cerebral ventricle. All cannulae were secured to the skull with dental cement and stainless steel screws. Each guide tube was fitted with an indwelling 23 ga stylet when not in use. Following surgery, each rat recovered for a period of 7-10 days.

Each rat was placed individually in a plastic cage for at least 2 hours before injection of the vasopressin antagonists or the control solution (sterile physiological saline). Febrile responses were evoked by infusing human purified interleukin-1 (Cistron; hpIL-1), by gravity flow, into a lateral cerebral ventricle (20µl hpIL-1

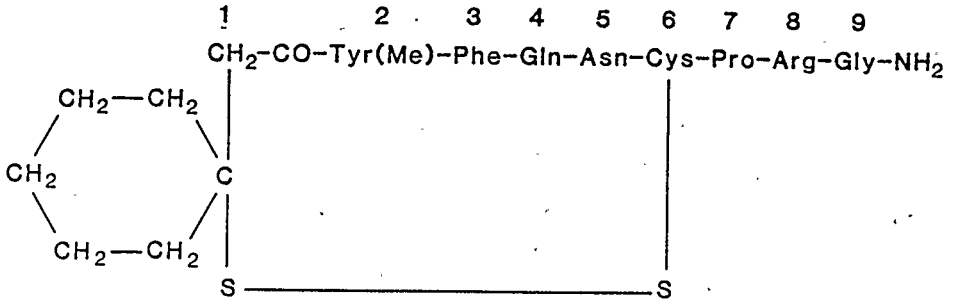
containing 20 units). Fifteen minutes before this, either saline (1.0 µl) or the vasopressin antagonists (200-400 pmoles) were injected bilaterally into the VSA over a 30-45 second period using a 27 ga injector needle affixed by PE-20 tubing to a Harvard infusion pump. The two vasopressin antagonists, 1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-(O-methyl)tyrosine arginine vasopressin and 1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-D-valine, 4-valine arginine vasopressin were supplied by Dr. M. Manning (Abbreviated as $d(CH_2)_5$Tyr(Me)AVP and $d(CH_2)_5$D-ValVAVP respectively; Figures 33 and 34). For control experiments hpIL-1 and heated hpIL-1 (90°C for 60 min) were infused into a lateral cerebral ventricle without any injection into the VSA. In addition, both the saline and vasopressin antagonists were injected into the VSA without a subsequent intracerebroventricular infusion of hpIL-1.

For 2 hours prior to and 3-5 hours following injections, the colonic temperature of each rat was monitored continuously using a thermistor probe (YSI 701) inserted 6 cm beyond the anus and taped to the tail. The temperature of each animal was recorded at 5-15 minute intervals on a digital datalogger (United Systems Digitec).

Upon completion of the experiments, each rat was anaesthetized deeply with sodium pentobarbital. The brain was then perfused with saline followed by formalin. The tissue was blocked in the coronal plane and sectioned at 50 µm on a sledge microtome. Each section was stained with neutral red and the injection sites localized by light microscopy.

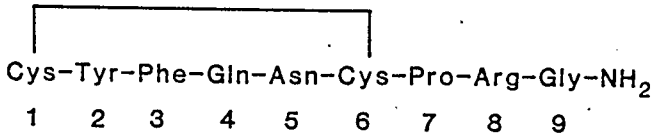



Arginine$^{8}$-Vasopressin (AVP)

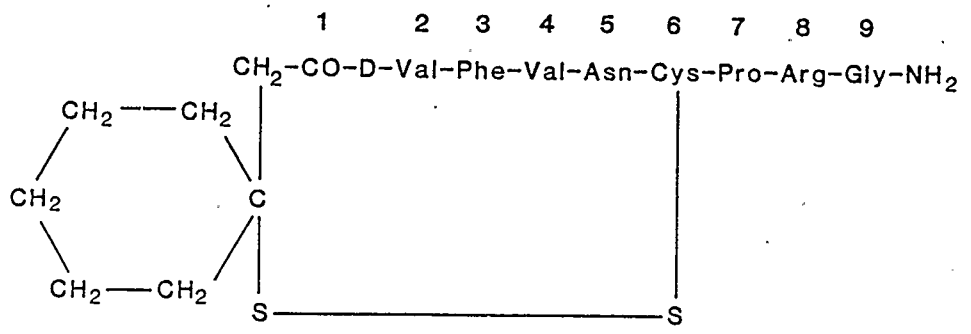



[1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-(O-methyl)tyrosine] arginine-vasopressin ($d(CH_2)_5$ Tyr(Me)AVP)

FIGURE 33 Structure of the vasopressin ($V_1$) antagonist.




Arginine$^{8}$-Vasopressin (AVP)




[1-(β-mercapto-β,-β-cyclopentamethylenepropionic acid), 2-D-valine, 4-valine] arginine-vasopressin ($d(CH_2)_5$-D-ValVAVP)

FIGURE 34 Structure of the vasopressin ($V_2$) antagonist.

Results were assessed statistically using an analysis of variance followed by the Scheffe post-hoc test. The fever indices were expressed as areas under the fever curves in °C.h.

3) Results

Intracerebroventricular (icv) administration of interleukin-1 (hpIL-1) evoked a rise in core temperature which was maximal 30-60 minutes after injection. This febrile action of hpIL-1 was abolished by heating the protein to 90°C for 60 minutes (Figure 35). When the icv administration of hpIL-1 was preceded by a bilateral injection of saline into the VSA, fever was produced which was not different from that induced by hpIL-1 alone (Figure 36). However, when the vasopressin ($V_1$) antagonist, $d(CH_2)_5Tyr(Me)AVP$, was injected similarly into the VSA 15 minutes prior to hpIL-1, a fever was evoked which was significantly greater in magnitude and duration. Figure 37 shows the effects of an intraseptal injection of a $V_1$ antagonist on the fever evoked by hpIL-1 along with the injection sites (see histological sites) in two individual animals. The effects of two different concentrations of $d(CH_2)_5Tyr(Me)AVP$ (200 and 400 pmoles bilaterally) are shown in Figure 38. The core temperature of these rats returned to baseline 8-10 hours following infusion of the hpIL-1 (data not shown). Figure 39 illustrates the thermoregulatory responses observed after bilateral injection of either saline or $d(CH_2)_5Tyr(Me)AVP$ (400 pmoles) into the VSA without subsequent icv administration of hpIL-1. Such injections evoked no significant alterations in the core temperature of the rats. In a different group of rats, the

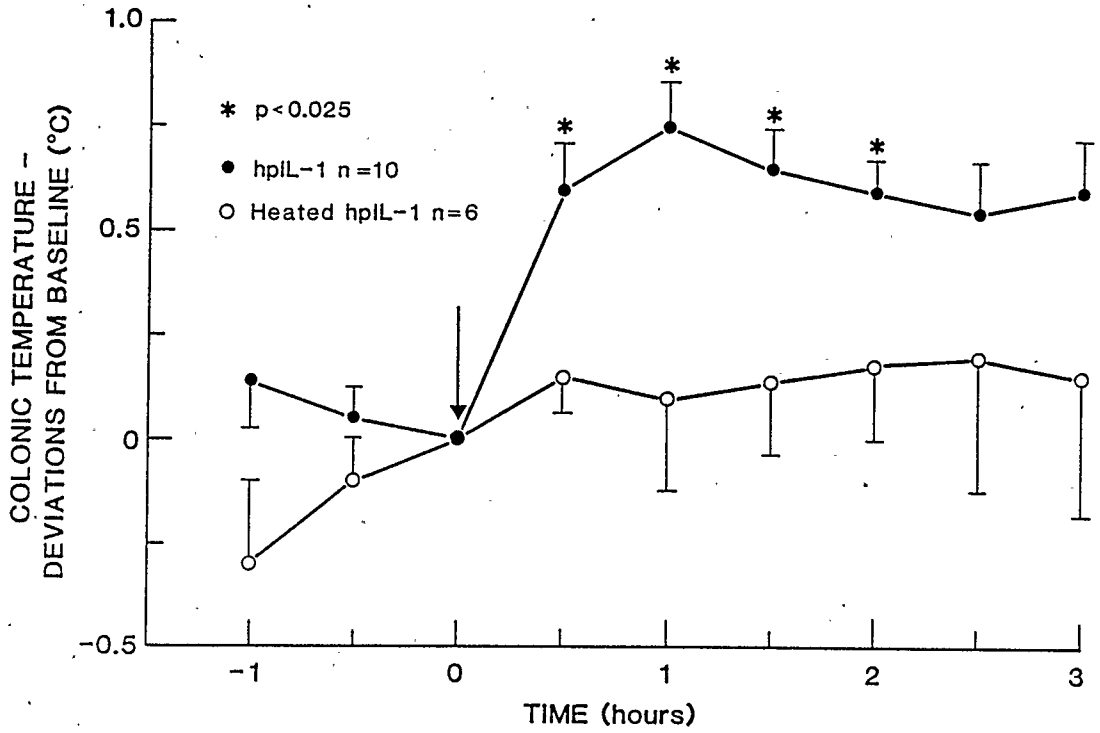



FIGURE 35 Mean temperature response (± SEM) to the intracerebroventricular (icv) infusion of interleukin-1 (hpIL-1; 20 units) or heated interleukin injected at arrow. (* $p < 0.025$)

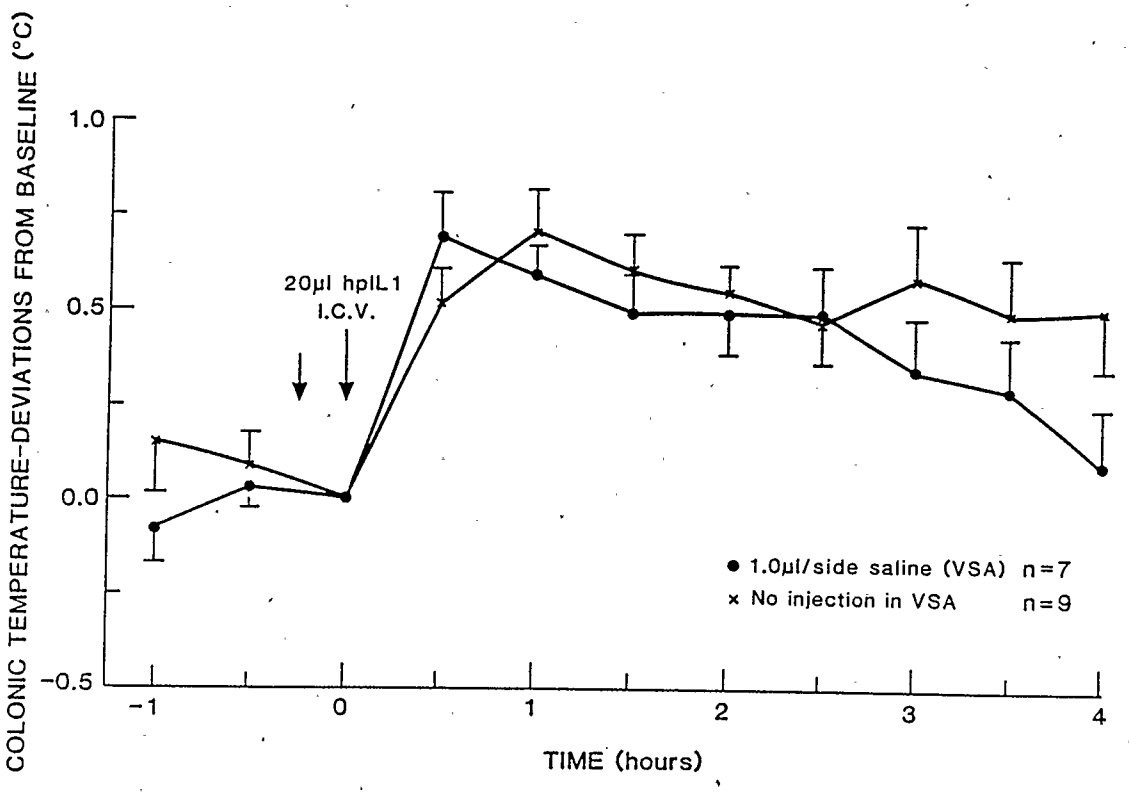



FIGURE 36 Mean temperature responses (± SEM) to the intracerebroventricular (icv) infusion of interleukin-1 (hpIL-1; 20 units). Fifteen minutes prior to this, saline was injected bilaterally into the ventral septal area (VSA; first arrow). There was no significant difference between the two groups.

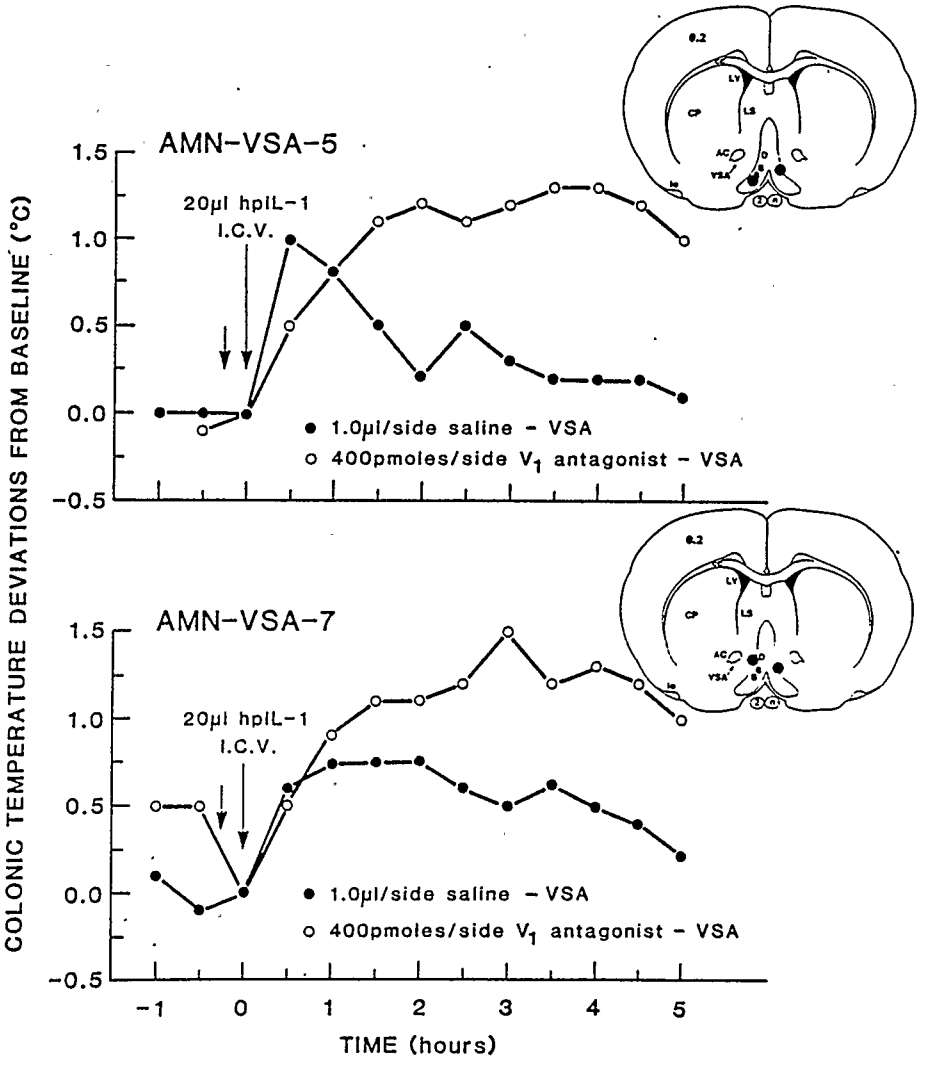
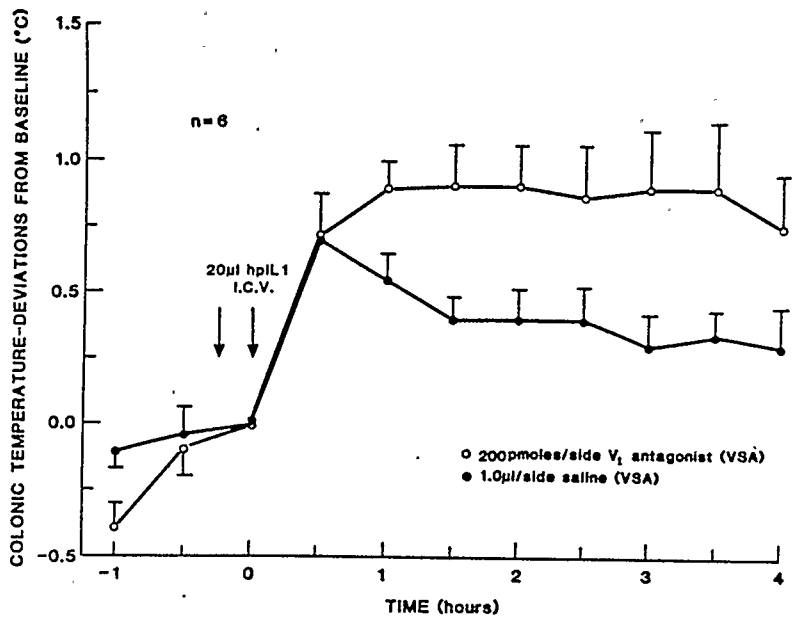
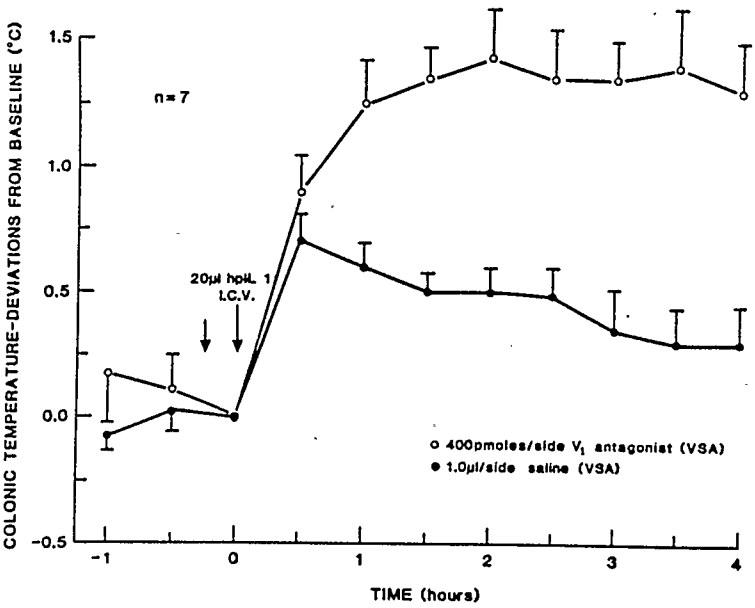



FIGURE 37 Temperature response of two individual rats (upper and lower) to the intracerebroventricular (icv) infusion of interleukin-1 (hpIL-1; 20 units. Fifteen minutes prior to this, either saline or the vasopressin ($V_1$ antagonist $d(CH_2)_5Tyr(Me)AVP$, were injected bilaterally into the ventral septal area (VSA; first arrow) in a concentration of 400 pmoles. For abbreviations see legend to Figure 42.

FIGURE 38 Upper panel: Mean temperature response (± SEM) to the intracerebroventricular (icv) infusion of interleukin-1 (hpIL-1; 20 units). Fifteen minutes prior to this, either saline or the vasopressin ($V_1$) antagonist $d(CH_2)_5Tyr(Me)AVP$, were injected bilaterally into the ventral septal area (VSA; first arrow) in a concentration of 200 pmoles.
Lower panel: Mean temperature response (± SEM) to the intracerebroventricular (icv) infusion of interleukin-1 (hpIL-1; 20 units). Fifteen minutes prior to this, either saline or the vasopressin ($V_1$) antagonist $d(CH_2)_5Tyr(Me)AVP$, were injected bilaterally into the ventral septal area (VSA; first arrow) in a concentration of 400 pmoles.


COLONIC TEMPERATURE-DEVIATIONS FROM BASELINE (°C)
1.5
1.0
0.5
0.0
-0.5
n=6
20µl hpIL1
I.C.V.
o 200pmoles/side V1 antagonist (VSA)
• 1.0µl/side saline (VSA)
-1
0
1
2
3
4
TIME (hours)


COLONIC TEMPERATURE-DEVIATIONS FROM BASELINE (°C)
1.5
1.0
0.5
0.0
-0.5
n=7
20µl hpIL 1
I.C.V.
o 400pmoles/side V1 antagonist (VSA)
• 1.0µl/side saline (VSA)
-1
0
1
2
3
4
TIME (hours)

vasopressin ($V_2$) antagonist, $d(CH_2)_5$-D-ValVAVP, was injected into the VSA. There was no alteration of the IL-1 induced fever when compared to the control response with saline (figure 40), or of the resting body temperature in afebrile rats (data not shown).

The fever indices obtained from the rats that were treated with $d(CH_2)_5$Tyr(Me)AVP were significantly greater than the controls with saline at both concentrations tested. In contrast, a similar injection of $d(CH_2)_5$-D-ValVAVP, prior to IL-1, produced fever which was not different from that in control experiments (figure 41).

The areas in the forebrain where injection of the $V_1$ antagonist enhanced IL-1 induced fever are indicated by closed circles (figure 42). Sites where injection of the antagonist did not alter the time course of IL-1 fever are indicated by open circles.

4) Discussion

Infusion of human purified interleukin-1 into a lateral ventricle of the rat evoked a fever. This effect was not obtained when heated interleukin-1 was used, suggesting that the rise in core temperature was unlikely to be due to contamination with endotoxin. The development and time course of the interleukin-1 fever was unaltered by an injection of saline into the VSA. However, when the $V_1$ antagonist, ($d(CH_2)_5$Tyr(Me)AVP), was injected similarly into the VSA (the area of the rat brain where vasopressin suppresses fever; see earlier) a fever was evoked which was significantly greater in magnitude and duration. In addition, this augmentation of the febrile response was dose-related. Initially (up to 30 mins), the rises in

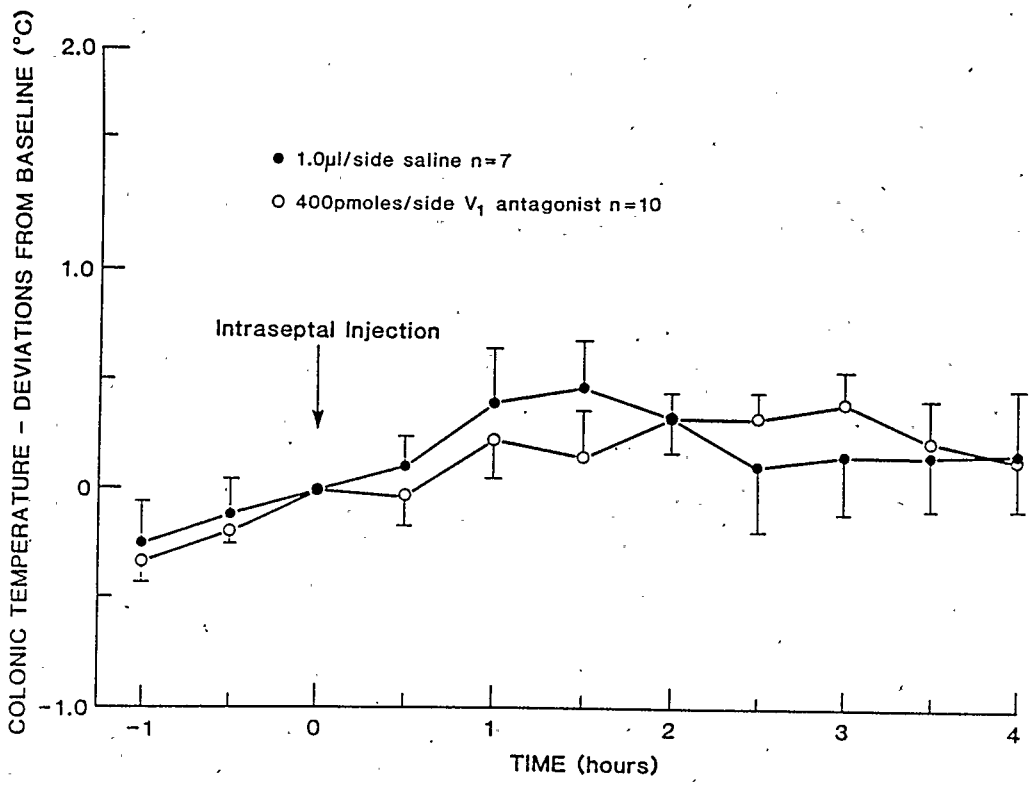



FIGURE 39 Mean temperature responses (± SEM) to the intracerebral injection of saline and vasopressin ($V_1$) antagonist into the ventral septal area. There was no significant difference between the two treatments.

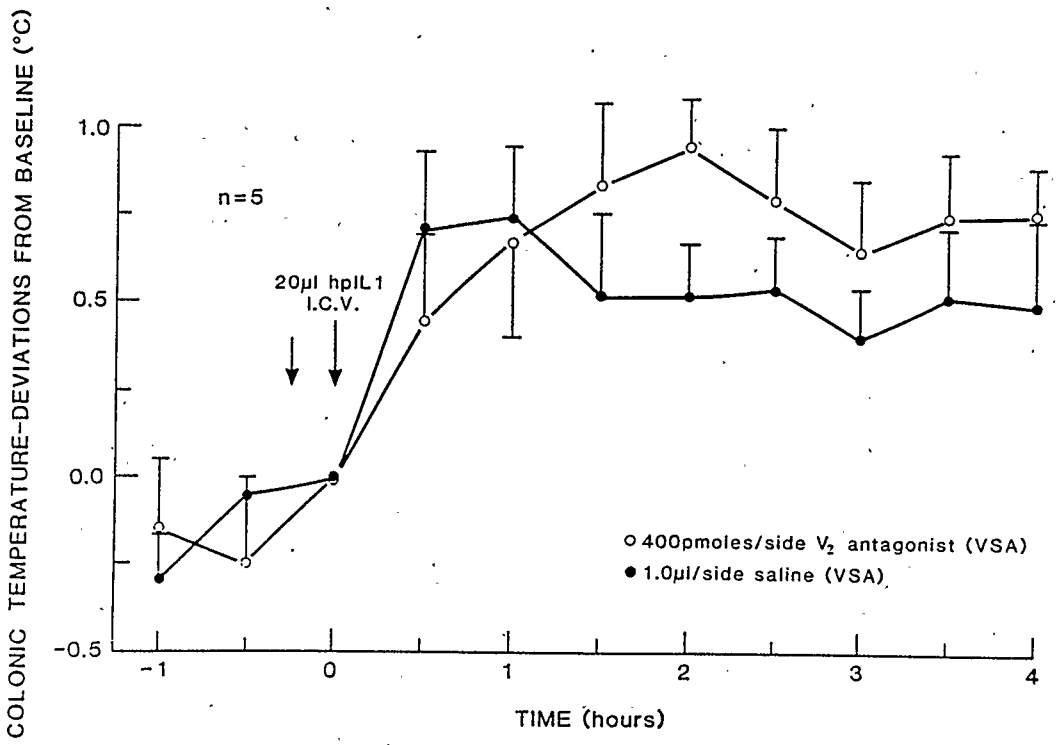



FIGURE 40 Mean temperature responses (± SEM) to the intracerebroventricular (icv) infusion of interleukin-1 (hpIL-1; 20 units). Fifteen minutes prior to this, either saline or the vasopressin ($V_2$) antagonist $d(CH_2)_5$-D-ValVAVP, were injected bilaterally into the ventral septal area (VSA; first arrow) in a concentration of 400 pmoles.

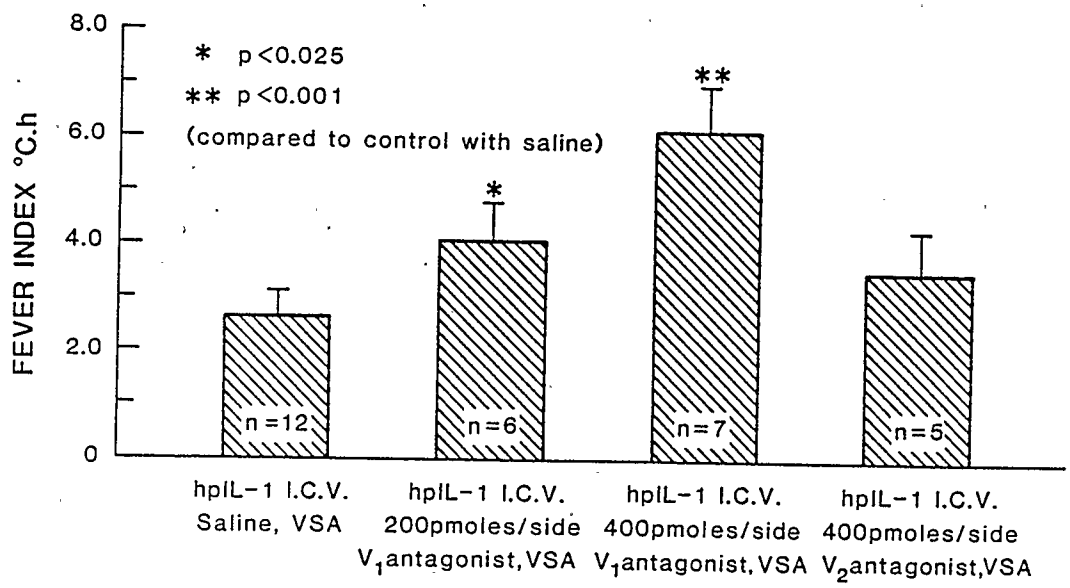



FIGURE 41 Effects of two vasopressin antagonists, injected into the ventral septum, on the febrile response evoked by intracerebroventricular interleukin-1. Both concentrations of $V_1$ antagonist (200 and 400 pmoles bilaterally) enhanced significantly the fever index over the control response with saline. In contrast, the $V_2$ antagonist (400 pmoles bilaterally) did not alter significantly the fever index from control values. (* $p < 0.025$; ** $p < 0.001$)

core temperature were similar between the saline and $V_1$ antagonist treated groups. However, instead of starting to return towards baseline levels like the controls with saline, the core temperatures of the $V_1$ antagonist treated rats remained elevated throughout the period of measurement. By five hours after injection of the interleukin-1, the body temperature of the antagonist treated rats had begun to return towards baseline levels. This relatively long duration of action for $d(CH_2)_5Tyr(Me)AVP$ has been reported previously for the antagonist (Kruszynski et al., 1980). The increase in fever does not appear to be due to a thermogenic action of the $V_1$ antagonist superimposed on the febrile effects of interleukin-1 because injection of the antagonist into the VSA, without interleukin-1, did not evoke any consistent thermoregulatory responses. Indeed, the thermoregulatory effects of intraseptal saline and $V_1$ antagonist are indistinguishable.

The vasopressin antagonist augmented fever when endogenous release of vasopressin from the septum has been postulated to be at its highest, since secretion of the peptide correlates negatively with febrile rises in body temperature (Cooper et al., 1979; Ruwe et al., 1985). Therefore, an explanation of these data may be that the $V_1$ antagonist, $d(CH_2)_5Tyr(Me)AVP$, prevents endogenously released vasopressin from interacting with its receptors in the VSA, thereby preventing AVP from limiting the magnitude of fever. In support of this, the $V_1$ antagonist that enhanced fever in this study has been reported previously to prevent the antipyretic action of exogenously administered vasopressin (Kasting and Wilkinson, 1986; see earlier). Further, receptors for vasopressin have been localized within the VSA

(Baskin et al., 1983) along with dense networks of immunoreactive vasopressin-like material (De Vries et al., 1985) composed almost entirely of AVP-containing terminals. It is possible also that the enhanced fevers are due to a non-specific action of the antagonist within the VSA since $d(CH_2)_5$Tyr(Me)AVP has been reported to have a neurodepressant action within the spinal cord (Porter and Brody, 1986). However, since another vasopressin antagonist, $d(CH_2)_5$-D-ValVAVP, did not enhance fever on injection into the VSA and in view of the other evidence supporting a role for AVP in fever suppression in the ventral septum, this is unlikely to explain completely these results. Since $d(CH_2)_5$Tyr(Me)AVP is a selective vasopressor antagonist, it is conceivable that the antipyretic effects of AVP are due to local changes in cerebral blood flow. However, since angiotensin II (a potent vasoconstrictor) does not modulate fever within the VSA (Kasting, 1980), this is unlikely.

The areas in the forebrain where injection of the $V_1$ antagonist enhanced fever were located predominantly within the VSA, an area of the brain bounded by the diagonal bands of Broca, ventral to the lateral septum. In addition to these findings, some sites were localized nearby in areas more rostral and dorsal to the majority. Areas in the forebrain where injection of the antagonist did not alter the time-course or duration of the febrile response were located more ventral and lateral to the sensitive sites, also in the lateral septum. Therefore, the areas in the brain where the $V_1$ antagonist enhanced fever are similar to those where exogenously administered vasopressin exerts its antipyretic action (see earlier). Similarly, where the vasopressin antagonist did not alter the characteristics of

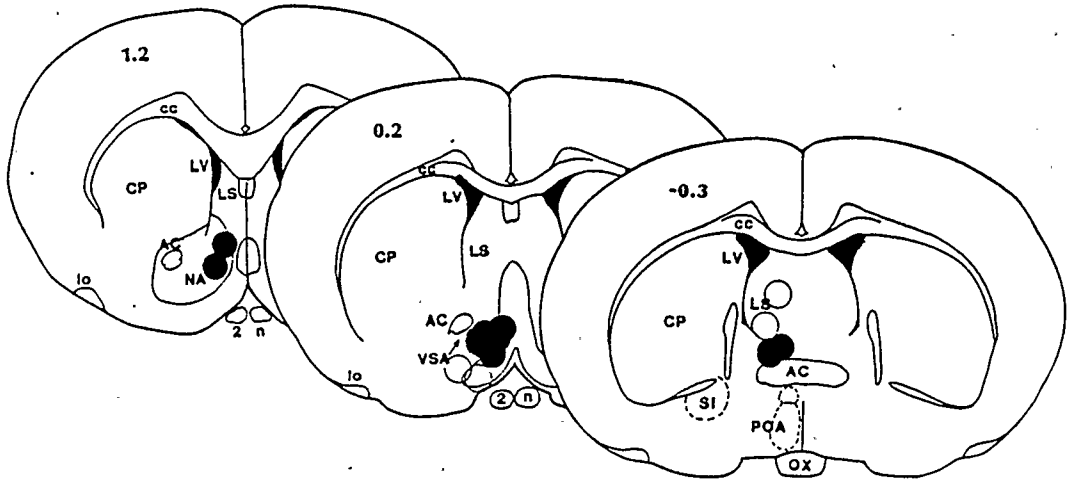



FIGURE 42 Schematic histological section depicting sites where the $V_1$ receptor antagonist enhanced interleukin-1 fever (closed circles). Areas where the antagonist did not alter interleukin-1 fever are also shown (open circles). Loci from both sides are indicated on the one side.
Abbreviations: AC, anterior commissure; cc, corpus callosum; CP, caudate putamen; lo, lateral olfactory tract; LS, lateral septum; LV, lateral ventricle; NA, nucleus accumbens; OX, optic chiasm; POA, preoptic area; SI, substantia innominata; VSA, ventral septal area; 2n, optic tracts.

interleukin-1 fever, exogenous AVP has little or no antipyretic effects, e.g. the lateral septum.

In contrast to the $V_1$ antagonist, intraseptal injection of the $V_2$ antagonist, $d(CH_2)_5$-D-ValVAVP, did not affect any characteristic of interleukin-1 fever at the concentration tested. This suggests that a central $V_2$ receptor is unlikely to be involved in the antipyretic effect of central vasopressin. In support of this view, a $V_2$ agonist, DDAVP, does not exert any antipyretic action against pyrogen fevers (see earlier).

It has been reported previously that perfusion of a specific AVP antibody in AVP-sensitive antipyretic sites in the sheep (Kasting, 1980) or rabbit (Malkinson et al., 1987) brain resulted in enhanced fevers. Our results extend these observations and, because of the nature of the antagonists, suggest that the receptor involved in the fever limiting properties of AVP may be similar to the vasopressor ($V_1$) subtype. Further, it can now be said that preventing the action of endogenously released vasopressin from reaching its receptor, either by sequestering it with an antibody or blocking it with an antagonist, effectively produces the same end result.

In conclusion, these data support and strengthen the hypothesis that AVP may function within the mammalian brain as an endogenous antipyretic. In addition, like other central effects of AVP, this action may be mediated by a central vasopressin receptor which resembles the peripheral $V_1$ subtype. However, these data do not exclude the possibility that AVP may exert its antipyretic action by interacting with a central receptor which is neither $V_1$ or $V_2$ in nature.

# VIII. MECHANISM OF VASOPRESSIN-INDUCED ANTIPYRESIS

## A. EFFECTS OF COOL AND WARM ENVIRONMENTS ON THE THERMOREGULATORY ACTIONS OF INTRASEPTAL VASOPRESSIN

### 1) Introduction

Although vasopressin suppresses pyrogen fever in all species tested so far, the mechanism of this antipyresis has not been determined. Vasopressin might influence core temperature during fever by 1) altering the set-point for body temperature control, 2) decreasing heat production and, 3) increasing heat loss, or a combination of all three. Experiments carried out thus far have provided no clear explanation of the mechanism of antipyretic action of vasopressin since AVP has not been injected directly into antipyretic-active sites in the VSA during various thermoregulatory challenges (i.e. over a number of ambient temperatures). Previous attempts to elucidate the mechanism(s) involved in the antipyretic action of vasopressin either have used the icv route of administration (Wilkinson and Kasting, 1986) or have not injected the peptide into AVP-sensitive antipyretic sites in the VSA (Banet and Wieland, 1985).

These experiments were undertaken to investigate the mechanism(s) involved in the antipyretic action of AVP by injecting the peptide into antipyretic-active sites in the VSA at cool (7°C) and warm (30°C) ambient temperatures.

2) Methods

Male Sprague-Dawley rats (280-350 g) were used for the experiments. They were maintained on a 12 hour light/dark cycle and food and water were available ad libitum.

Stainless-steel guide cannulae were implanted bilaterally above the VSA as outlined previously. In addition, a jugular catheter was implanted and exteriorized at the back of the neck so that intravenous injections of pyrogen could be made into the conscious and unrestrained rat. The EP used to produce fever was prepared and harvested from rabbit peritoneal exudates and was purified partially using ultrafiltration. Rats recovered from surgery for a period of 7 days.

Injections into the VSA, measurement of colonic temperature and histological site verification were carried out as described previously. Results were assessed statistically using an analysis of variance followed by the Scheffe post-hoc test.

3) Results

Intravenous injection of EP evoked a prompt rise in core temperature (Figures 43 and 44) which could be abolished if the solution was heated (data not shown). If the iv injection of pyrogen was preceded immediately by a bilateral injection of AVP (0.5 pmoles) into the VSA, fever was suppressed for 15 minutes (Figures 43 and 45). However, in other rats, similar injections of the peptide into nearby areas of the brain (around the anterior commissure and lateral septum)

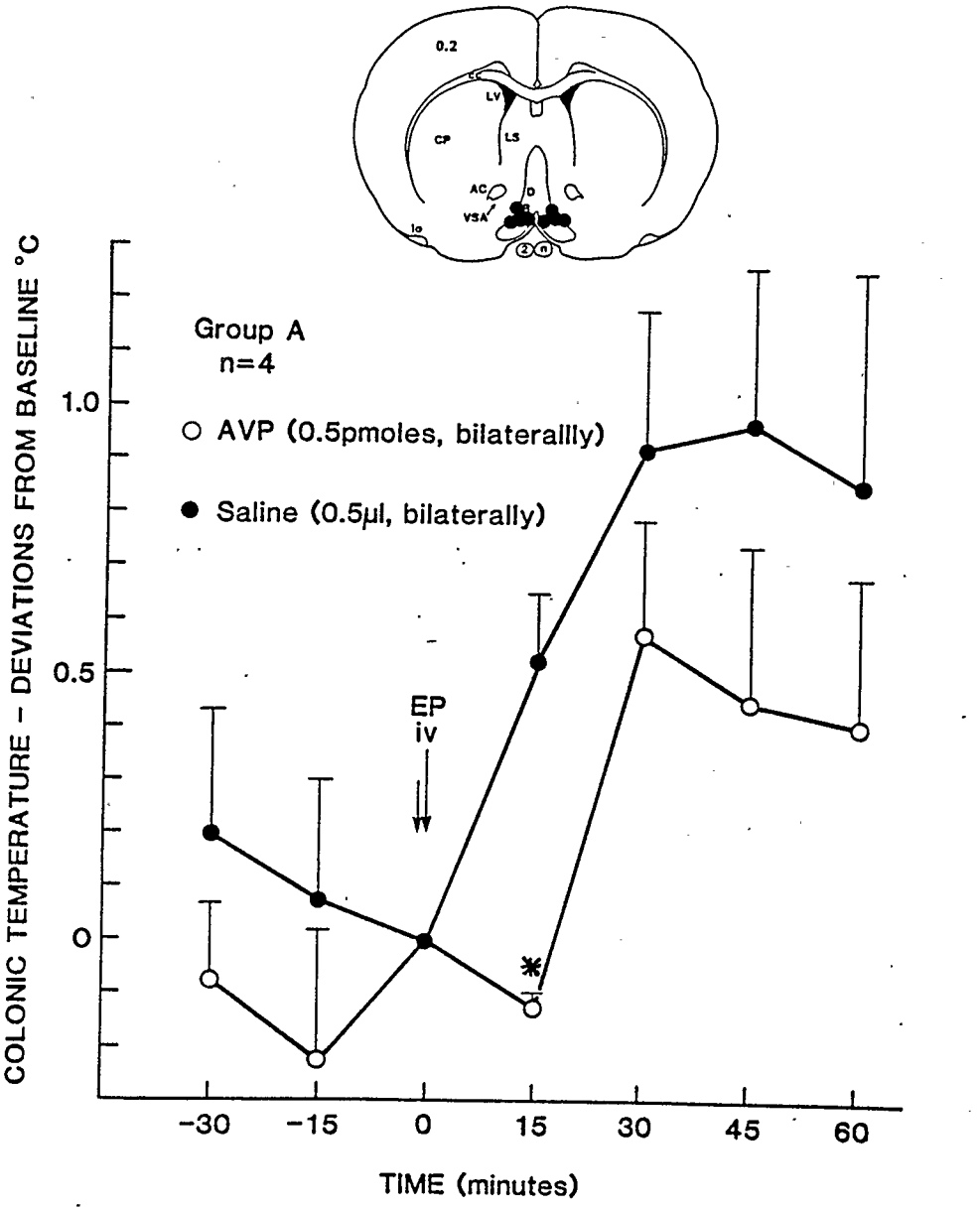



Figure 43

Mean colonic temperature responses (± SEM; Group A) to the intravenous (iv) injection of crude endogenous pyrogen (EP). Five minutes prior to this, either saline (0.5 µl) or vasopressin (0.5 pmole/0.5 µl) were injected bilaterally into the ventral septal area. Vasopressin suppressed significantly the febrile response for 15 minutes. (* $p < 0.01$). Loci of injections are indicated by closed circles in the histological inset. For abbreviations, see the legend for figure 42.

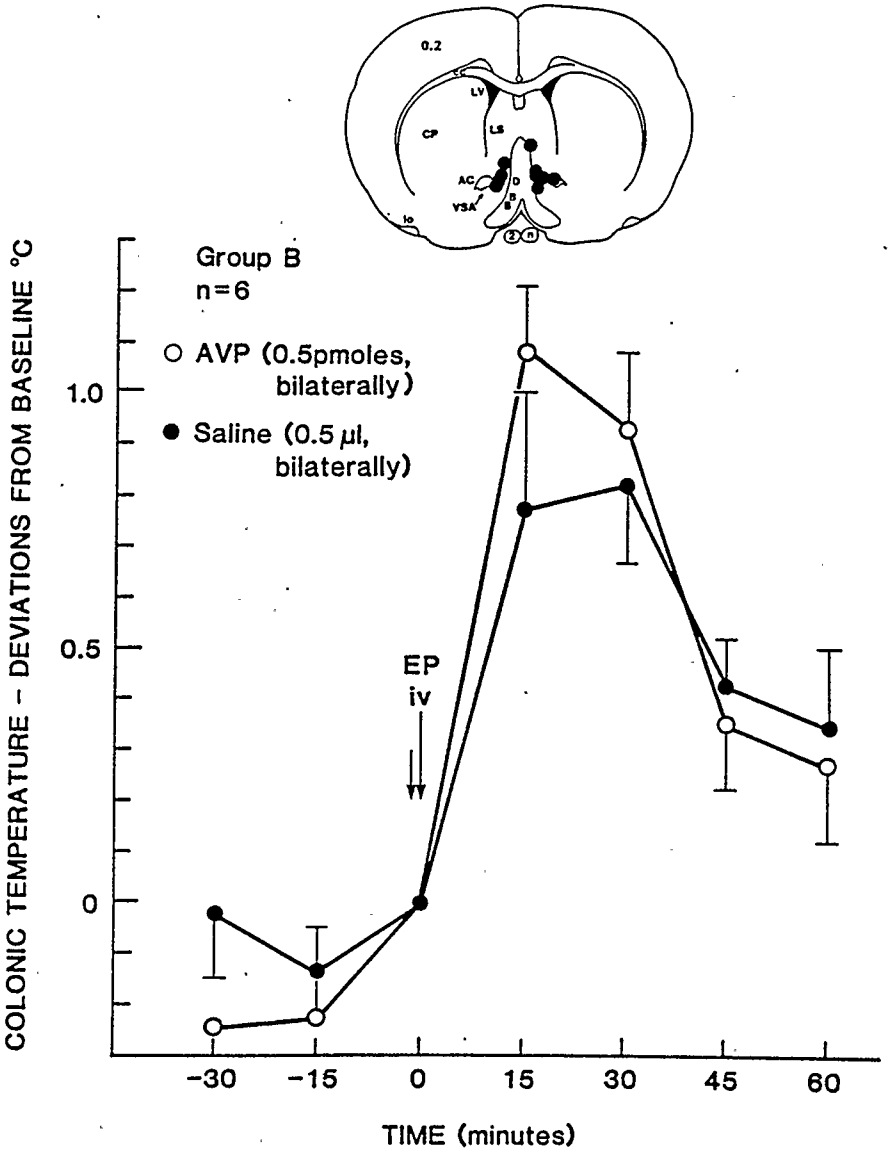



Figure 44

Mean colonic temperature responses (± SEM; Group B) to the intravenous (iv) injection of crude endogenous pyrogen (EP). Five minutes prior to this, either saline (0.5 µl) or vasopressin (0.5 pmole/0.5 µl) were injected bilaterally into areas outside the ventral septal area. Vasopressin did not alter the normal development of the febrile response. Loci of injections are indicated by closed circles in the histological inset. For abbreviation see the legend for figure 42.

did not attenuate fever when compared to the control responses with saline (Figures 44 and 46). The rats were divided into two groups: group A, containing those rats which responded to intracerebral AVP with a suppression of fever and, group B, containing those rats which did not respond to intracerebral AVP. Both groups of rats received another bilateral injection of AVP (0.5 pmoles in 0.5 µl) at each ambient temperature (7°C and 30°C) in the absence of pyrogen. Under these conditions, intraseptal AVP evoked thermoregulatory effects that were similar between group A and B at both ambient temperatures (Figure 46).

4) Discussion

Bolus bilateral injection of 0.5 pmoles AVP into the VSA suppressed fever for approximately fifteen minutes. In agreement with previous reports, this action of vasopressin was site specific.

The remainder of the experiments were undertaken to determine some aspects of the mechanism of the antipyretic response. If vasopressin were acting to reduce febrile body temperatures by preventing heat production/heat conservation, it might be expected that injection of AVP into the VSA at 7°C might evoke a fall in core temperature. This is because the rats would be recruiting heat producing/conserving mechanisms at this ambient temperature as it is well below thermoneutrality. Alternatively, if vasopressin stimulated heat loss during febrile episodes, this might explain the fever reducing properties of the peptide. These data reveal that injection of AVP into the VSA or into nearby sites (at ambient temperatures of

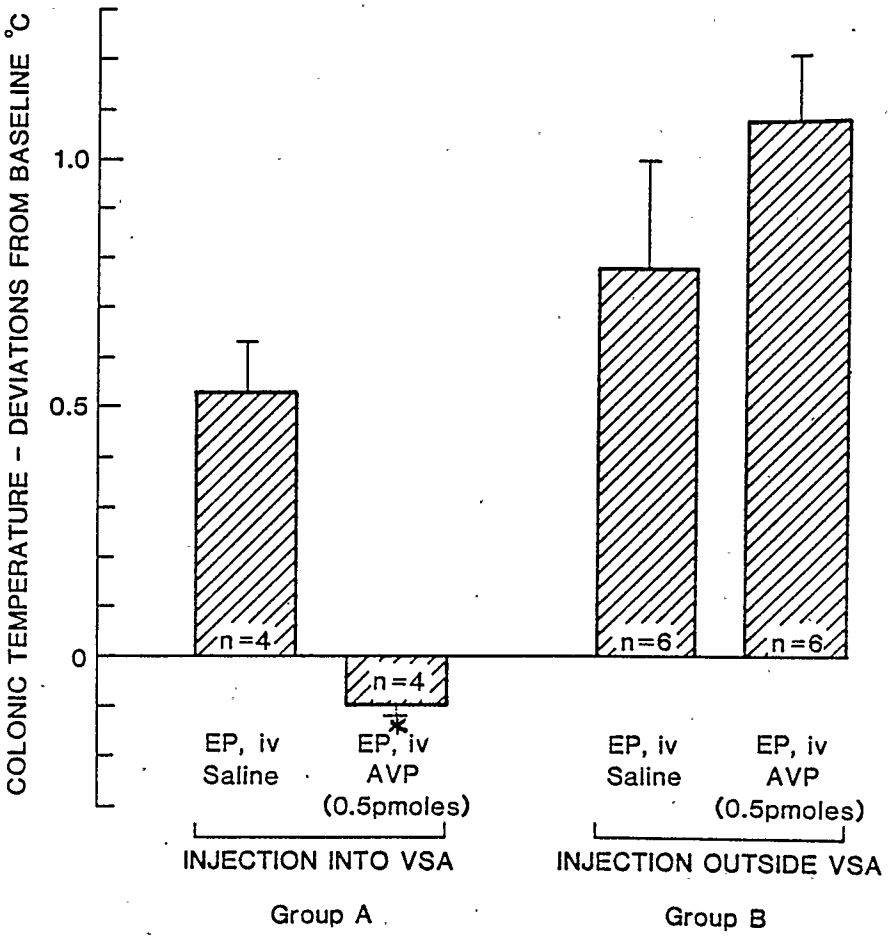



Figure 45 Mean colonic temperature responses (± SEM) 15 minutes following an intravenous (iv) injection of crude endogenous pyrogen (EP) in group A and group B. Five minutes prior to the EP injection, AVP (0.5 pmole/0.5 $\mu$l) was injected bilaterally into the forebrain (* $p < 0.01$)

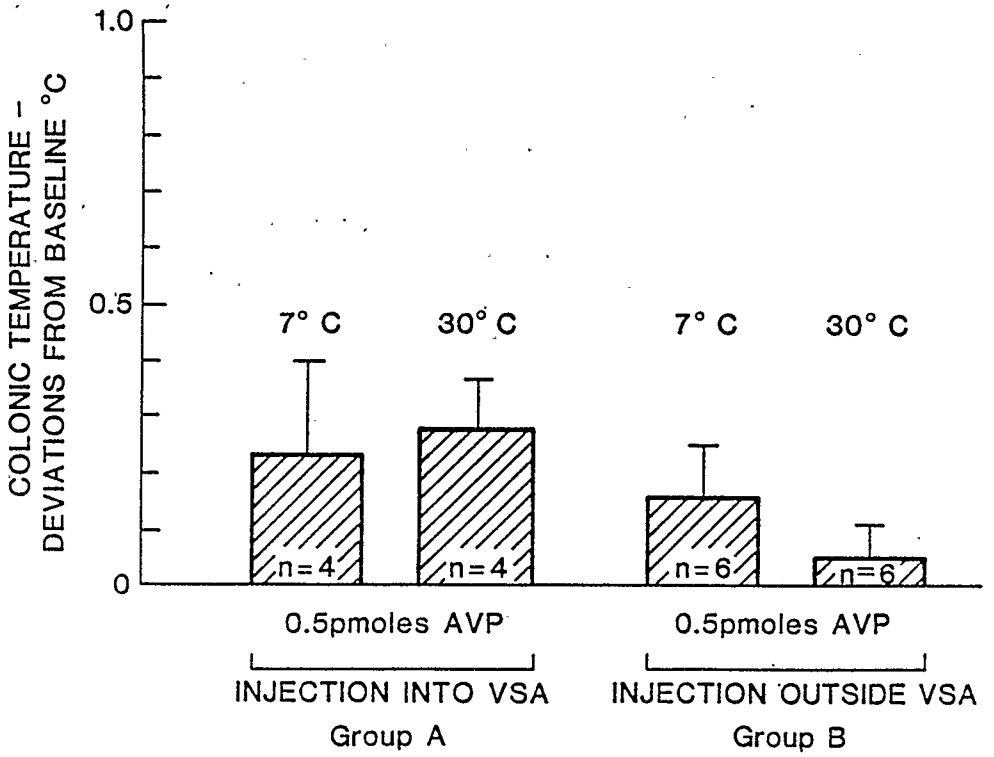



Figure 46

Mean colonic temperature responses (± SEM) 15 minutes following an intracerebral injection of AVP (0.5 pmole/0.5 µl) in groups A and B at 7°C and 30°C.

7°C and 30°C) did not produce temperature changes that were different. Therefore, it is unlikely that vasopressin suppresses fever by inhibiting heat production or by stimulating heat loss, rather, it is possible that intraseptal AVP affects 'set-point'. These results are in partial agreement with those of Wilkinson and Kasting (1986) but are the opposite of those described by Banet and Wieland (1985). An explanation of this discrepancy may be that the latter workers injected vasopressin into antipyretic-inactive sites in the dorsal septum at a concentrations 2000 times those used in this study.

In conclusion, exogenously administered vasopressin may exert its antipyretic action by changing the set-point such that it is reset during fever.

# IX. GENERAL DISCUSSION AND CONCLUSIONS

The aim of the work reported in this thesis was to investigate further the involvement of vasopressin as an antipyretic and to provide evidence that this peptide might function as a neurotransmitter within the VSA concerned with endogenous antipyresis. In addition, it was intended to address the discrepant observations regarding the antipyretic effect of vasopressin in the rabbit and the hyperthermic action of the peptide in the rat.

Perfusion of vasopressin within the ventral septum of a number of species, including the rabbit, rat, cat and guinea-pig, suppressed the fever evoked by the peripheral or central injection of pyrogens in a dose-related manner. Thus, central administration of vasopressin suppressed the fever evoked by endotoxin, interleukin-1 and prostaglandin $E_2$. The antipyretic action of vasopressin, which may involve an alteration in 'set-point', was observed only when vasopressin was perfused or injected within a discrete area of the brain. When AVP was administered outside the VSA the normal development of the febrile response was unaffected. This may explain, in part, the discrepant observations regarding the antipyretic action of vasopressin in the rabbit reported earlier. In the rat, the intracerebroventricular injection of vasopressin suppressed fever at concentrations well below those required to induce hypothermia. In contrast, similar injection of vasopressin into the rabbit did not result in any suppression of fever. These data suggest that precise physiological roles for peptides are unlikely to be determined if only the ventricular route of administration is used to investigate their

actions. Indeed, to understand fully the thermoregulatory actions of vasopressin requires a careful neuroanatomical evaluation of the effects of the peptide on the thermoregulatory system. In this regard, vasopressin exerts a very different action dependent on whether it is administered into a lateral cerebral ventricle (hypothermia or antipyresis depending on dose), the preoptic area (hyperthermia) or the ventral septal area (antipyresis).

The antipyretic action of vasopressin demonstrated peptide specificity in that the structurally similar neurohypophyseal peptide, oxytocin, did not exert any antipyretic activity when perfused in brain sites where vasopressin suppressed fever. This suggests that vasopressin does not exert its effects by interacting with an oxytocin receptor in the VSA. Further studies on the receptor involved in the antipyretic effect of vasopressin shows that it resembles the peripheral $V_1$ subtype of vasopressin receptor. This is because a $V_1$ antagonist, $d(CH_2)_5$Tyr(Me)AVP, can prevent the antipyretic effect of exogenously administered vasopressin whereas a $V_2$ agonist, DDAVP, is devoid of antipyretic activity against pyrogen fever.

The role of endogenous vasopressin in fever suppression was investigated using vasopressin antagonists. Two antagonists, relatively specific to the $V_1$ and $V_2$ subtype of peripheral vasopressin receptor, were injected into the area of the brain where vasopressin suppresses fever. A $V_1$ antagonist [$d(CH_2)_5$Tyr(Me)AVP], but not a $V_2$ antagonist [$d(CH_2)_5$D-ValVAVP], on injection into the VSA enhanced the febrile response to a pyrogen challenge. In the case of the $V_1$ antagonist, fever was enhanced at the time when endogenous release of vasopressin from the VSA has been postulated to be at its highest

since secretion of the neuropeptide correlates negatively with febrile changes in body temperature (Cooper et al., 1979). Therefore, the $V_1$ antagonist, by blocking vasopressin receptors in the VSA prevented endogenously released vasopressin from limiting the magnitude of fever. In accordance with the site specificity of the antipyretic effect of exogenous vasopressin, the $V_1$ antagonist enhanced fever only when injected into AVP-sensitive antipyretic sites in the VSA. For example, injections of the antagonist into the dorsal septum did not enhance fever. The specificity of vasopressin actions within the VSA are supportive of a neurotransmitter role for this peptide. Further, since an antagonist can block similarly the effect of exogenous administration and endogenous release of vasopressin, an important criteria necessary to validate a transmitter candidate has been demonstrated.

The question arises as to what is the significance of a system of endogenous antipyresis? Clearly, the system is not intended to abolish febrile rises in body temperature since these are observed commonly. The available evidence (on the release of AVP from the VSA and blockade of AVP actions in the VSA during fever) suggests that vasopressin may be important in the latter stages of fever, in the return toward prefebrile body temperatures. In addition, vasopressin may act in septal sites to regulate fever within 'beneficial levels' since preventing the action of endogenously released AVP results in enhanced fevers. Unfortunately, the data relating to the significance of fever and its contribution to host-defence are conflicting and inconclusive. Therefore, the statement that vasopressin acts to keep febrile body temperatures moderate (and that this is beneficial) is

based on speculation rather than experimental fact. However, it is well known that excessive rises in body temperature can result in permanent CNS damage and, in young children, febrile convulsions, therefore, there would be some benefit to modulating febrile rises in body temperature. Whether an interaction (abnormal or otherwise) of vasopressin with neurons in the VSA is involved in the pathogenesis of febrile convulsions remains to be determined also.

Another important question relating to the significance of vasopressin and endogenous antipyresis is its relation to the absence of fever observed at term in guinea-pigs and in some strains of sheep. Direct evidence supporting the involvement of AVP in the suppression of fever observed at term exists for the guinea-pig. Therefore, insights into the significance of a system of endogenous antipyresis may be obtained from the potential benefits of a period where fever is absent at term. Fever at parturition may compromise the maternal-newborn bond thereby resulting in rejection by the mother. In addition, the maturation of lung surfactant (which is important for respiratory function) is affected adversely at febrile temperatures. These are two instances where a negative modulator of fever would be of potential benefit. Whatever the importance, the central mechanisms involved in the antipyretic response observed at term required further investigation.

Kasting et al. (1982) have proposed that vasopressin may act in a number of different ways, both peripherally and centrally, to restore homeostasis during infection and fever. This is an attractive hypothesis which takes into account what is known currently about vasopressin and its actions. Thus, further work should focus on the

complex interactions that occur during fever and not just on fever itself. In addition, the potential interaction of other peptide systems (e.g. those involving α-MSH) may enable a clearer understanding of how the CNS responds during fever.

Whatever the significance of vasopressin's antipyretic action there is still much work required before it can be shown conclusively that vasopressin acts within the VSA as a neurotransmitter during fever, specifically the effects of AVP on VSA neurons during fever have not been elucidated. Such investigations may provide much needed information on the mechanisms involved in vasopressin induced antipyresis, i.e. when and how is the system activated/deactivated, and under what circumstances? These events then could be correlated with the effects on VSA neurons of exogenously administered vasopressin. It has been demonstrated by Disturnal (1986) that vasopressin may act as a neuromodulator within the VSA but intracellular studies to determine the neurophysiology of such actions have yet to be done. Another technique which may be used to investigate the mechanisms underlying vasopressin-induced antipyresis is "in situ" hybridization. This procedure allows the direct examination of the effects of physiological manipulations on the expression of neurotransmitter messenger RNA in specific areas of the brain, in this case the expression of vasopressin mRNA in the BST or PVN (putative sources of vasopressin to the VSA). This relatively new technique may provide much needed information on the time-course of activation of the vasopressinergic pathways(s) involved in endogenous antipyresis. Such investigations may serve to answer questions concerning the mechanisms of endogenous

antipyresis and on the CNS control of thermoregulatory processes under both febrile and afebrile conditions.

## X. REFERENCES


1. Aarden, L.A., Brunner, T.K., Cerottini, J.C., Dayer, J.M., de Week, A.L., Dinarello, C.A., Sabato, G.O., Farrar, J.J., Gery, I., Gillis, S., Handschumacher, R.E. Henney, C.S., Hoffman, M.K., Koopman, W.J., Krane, S.M., Lachman, L.B., Lefkowits, I., Mishell, R.E. Mizel, S.B., Oppenheim, J.J., Paetkav, V., Plate, J., Rollinghoff, M., Rosenstreich, D., Rosenthal, A.S., Rosenwasser, L.J. Schimpl., A., Schim, H.S., Simon, P.L., Smith, D.A., Wagner, H., Watson, J.D., Wecker, E. and Wood, B.D. (1979). Revised nomenclature for antigen non-specific T cell proliferation and helper factors. J. Immunol. 123: 2928-2929 (letter).

2. 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.

3. Abood, L.G., Knapp, R., Mitchell, T., Booth, M. and Schwab, L. (1980). Chemical requirements of vasopressins for barrel rotation convulsions and reversal by oxytocin. J. Neurosci. Res. 5: 191-199.

4. Adair, E.R. (1974). Hypothalamic control of thermoregulatory behavior: preoptic posterior hypothalamic interaction. In: Recent studies of hypothalamic function (K. Lederis and K.E. Cooper, eds.) Karger, Basel, pp. 341-358.

5. Albers, H.E., Pollock, J., Simmons, W.H. and Ferris, C.F. (1986). A $V_1$-like receptor mediates vasopressin-induced flank marking behavior in hamster hypothalamus. J. Neurosci. 6: 2085-2089.

6. 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.

7. Alexander, S.J., Cooper, K.E. and Veale, W.L. (1987). Blockade of prostaglandin $E_1$ hyperthermia by sodium salicylate given into the ventral septal area of the rat brain. J. Physiol. 384: 223-231.

8. Alexander, G. and Williams, D. (1968). Shivering and non-shivering thermogenesis during summit metabolism in young lambs. J. Physiol. 198: 251-276.

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

10. Andersson, B. and Leskell, L.G. (1975). Effects on fluid balance of intraventricular infusions of prostaglandin $E_1$. Acta. Physiol. Scand. 93: 286-288.

11. Aronsohn, E. and Sachs, J. (1885). Die Beziehungen des Gehirns zur Körperwärme und zum Fieber. Pflügers Arch. 37: 232-300. (from Lomax, 1979).

12. Artunkel, A.A., Marley, E. and Stephenson, J.D. (1977). Some effects of prostaglandin $E_1$ and $E_2$ injected into the hypothalamus of young chicks: Dissociation between endotoxin fever and the effects of prostaglandins. Br. J. Pharmacol. 61: 39-46.

13. 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.

14. Atkins, E. and Wood, W.B. (1955). Studies on the pathogenesis of fever. II. Identification of an endogenous pyrogen in the blood stream following the injection of typhoid vaccine. J. Exp. Med. 102: 499-516.

15. Atkins, E., Allison, F., Smith, M.R. and Wood, W.B. (1955). Studies on the antipyretic action of cortisone in pyrogen-induced fever. J. Exp. Med. 101: 353-366.

16. Audigier, S. and Barberis, C. (1985). Pharmacological characterization of two specific binding sites for neurohypophyseal hormones in hippocampal synaptic plasma membranes of the rat. EMBO J. 4: 1407-1412.

17. 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.

18. Auron, P.E., Webb, A.C., Rosenwasser, L.J., Mucci, S.F., Rich, A., Wolff, S.M. and Dinarello, C.A. (1984). Nucleotide sequence of human monocyte interleukin-1 precursor cDNA. Proc. Natl. Acad. Sci. U.S.A. 81: 7907-7911.

19. Azzaroni, A., Cevaloni, D., Ferrari, G. and Parmeggiani, P.L. (1985). Thermosensitive neurones during sleep in cats. J. Physiol. 369; 60P. (Abstract)

20. Baird, J.A., Hales, J.R.S. and Lang, W.J. (1974). Thermoregulatory responses to the injection of monoamines, acetylcholine and prostaglandins into the lateral cerebral ventricle of the echidna. J. Physiol. 236: 539-548.

21. Banet, M. (1979). Fever and survival in the rat. The effect of enhancing fever. Pflügers Arch. 381: 35-38.

22. Banet, M. (1986). Fever in mammals: Is it beneficial? Yale J. Biol. Med. 58: 117-124.

23. Banet, M. and Wieland, U.E. (1985). The effect of intraseptally applied vasopressin on thermoregulation in the rat. Brain Res. Bull. 14: 113-116.

24. Baracos, V., Rodemann, H.P., Dinarello, C.A. and Goldberg, A.L. (1983). Stimulation of muscle protein degradation and prostaglandin $E_2$ release by leukocytic pyrogen (interleukin-1). A mechanism for the increased degradation of muscle protein during fever. N. Engl. J. Med. 308: 553-558.

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

26. 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.

27. Baskin, D.G., Petracca, F. and Dorsa, D.M. (1983). Autoradiographic localization of specific binding sites for [$^3$H] [Arg$^8$] vasopressin in the septum of the rat brain with tritium-sensitive film. Eur. J. Pharmacol. 90: 155-157.

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

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

30. Belyavskii, E.M. and Abromova, E.L. (1975). Effect of leucocyte pyrogen on thermosensitive neurons in the anterior hypothalamus. Bull. Eksp. Biol. Med. 80: 17-20. (from Eisenman, 1982).

31. Bennett, 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.

32. Benson, M.D., Aldo-Bensen, M.A., Shirahama, T., Borel, Y. and Cohen, A.S. (1975). Suppression of in vitro antibody response by a serum factor (SAA) in experimentally induced amyloidosis. J. Exp. Med. 142: 236-241.

33. Berl, T. and Schrier, R.W. (1973). Mechanism of the effect of prostaglandin $E_1$ on renal water excretion. J. Clin. Invest. 52: 463-471.

34. Bernard, C. (1876). Leçons sur la chaleur animâle, sur les effets de la chaleur et sur la fièvre. J.B. Bailliere et Fils, Paris. (from Lomax, 1979).

35. 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.

36. Bernheim, H.A. and Dinarello, C.A. (1985). Effect of protein synthesis inhibitors on leukocytic pyrogen-induced in vitro hypothalamic prostaglandin production. Yale J. Biol. Med. 58: 179-187.

37. 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.

38. 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.

39. Biegon, A., Terlou, M., Voorhuis, Th.D. and DeKloet, E.R. (1984). Arginine-vasopressin binding sites in the rat brain: a quantitative autoradiographic study. Neurosci. Lett. 44: 229-234.

40. Blatteis, C.M. (1975). Postnatal development of pyrogen sensitivity in guinea pigs. J. Appl. Physiol. 39: 251-257.

41. Blatteis, C.M. (1977). Comparison of endotoxin and leukocytic pyrogen pyrogenicity in newborn guinea-pigs. J. Appl. Physiol. 42: 355-361.

42. Blatteis, C.M. (1986). Fever: Is it beneficial? Yale J. Biol. Med. 59: 107-116.

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

44. Blatteis, C.M., Hunter, W.S., Llanos, J., Ahokas, R.A. and Mashburn, T.A. (1984). Activation of acute phase responses by intrapreoptic injections of endogenous pyrogen in guinea-pigs. Brain Res. Bull. 12: 689-695.

45. Blatteis, C.M. and Smith, K.A. (1979). Hypothalamic sensitivity to leucocytic pyrogen of adult and newborn guinea-pigs. J. Physiol. 296: 177-192.

46. Bligh, J. and Milton, A.S. (1973). The thermoregulatory effects of prostaglandin $E_1$ when infused into the lateral cerebral ventricle of the Welsh Mountain Sheep at different ambient temperatures. J. Physiol. 229: 30-31P. (Abstract)

47. Bligh, J., Silver, A. and Smith, C.A. (1977). A central cholinergic synapse between cold sensors and heat production effectors in the sheep: True or false? J. Physiol. 266: 88-89P. (Abstract)

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

49. 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.

50. Brodie, B. (1811). Some physiological researches respecting the influence of the brain on the actions of the heart, and on the generation of animal heat. Philos. Trans. R. Soc. Lond. (Biol.) 36-48. (from Lomax, 1979).

51. Brodie, B. (1812). Further experiments and observations on the influence of the brain on the generation of animal heat. Philos. Trans. R. Soc. Lond. (Biol.) 378-393. (from Lomax, 1979).

52. 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: PC852-853 (Abstract).

53. Brown, M., Ling, N. and Rivier, J. (1981). Somatostatin-28, somatostatin-14 and somatostatin analogs: Effects on thermoregulation. Brain Res. 214: 127-135.

54. Buijs, R.M. and Swaab, D.F. (1979). Immunoelectron-microscopical demonstration of vasopressin and oxytocin in the limbic system of the rat. Cell Tiss. Res. 204: 355-365.

55. 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 Tiss. Res. 186: 423-433.

56. Buijs, R.M. and Van Heerikhuize, J.J. (1982). Vasopressin and oxytocin release in the brain - a synaptic event. Brain Res. 252: 71-76.

57. Burbach, J.P.H. and Lebouille, J.L.M. (1983). Proteolytic conversion of arginine vasopressin and oxytocin by brain synaptic membranes. J. Biol. Chem. 258: 1487-1494.

58. Burnard, D.M., Pittman, Q.J. and Veale, W.L. (1983). Increased motor disturbances in response to arginine vasopressin following hemorrhage or the administration of hypertonic saline: Evidence for central AVP release in the rat. Brain Res. 273: 59-65.

59. 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: 40-46.

60. Cabanac, M., Duclaux, R. and Gillet, A. (1970). Thermoregulation comportementale chez le chien. Effet de la fièvre et de la thyroxine. Physiol. Behav. 5: 697-704.

61. 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.

62. 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.

63. Cannon, J.G. and Dinarello, C.A. (1985). Increased plasma interleukin-1 activity in women after ovulation. Science 227: 1247-1249.

64. Cantor, H.S. and Hampton, M. (1978). Indomethacin in submicromolar concentrations inhibits cyclic AMP-dependent protein kinase. Nature 276: 841-842.

65. Carlisle, H.J. (1969). Effect of preoptic anterior hypothalamic lesions on behavioral thermoregulation in cold. J. Comp. Physiol. Psychol. 69: 391-402.

66. Carr, D.B., Bergland, R., Hamilton, A., Blume, H., Kasting, N., Arnold, M., Martin, J.B. and Rosenblatt, M. (1982). Endotoxin stimulated opioid peptide secretion: Two secretory pools and feedback control in vivo. Science 217: 845-848.

67. Chossat, C. (1820). Extrait d'un memoire sur l'influence du système nerveux sur la chaleur animâle. Ann. Chim. Phys. 15: 37-49. (from Lomax, 1979).

68. Chowers, I., Conforti, N. and Feldman, S. (1968). Local effects of cortisol in the preoptic area on temperature regulation. Am. J. Physiol. 214: 538-542.

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

70. Clark, W.G. and Cumby, H.R. (1976). Antagonism by antipyretics of the hyperthermic effect of a prostaglandin precursor, sodium arachidonate, in the cat. J. Physiol. 257: 581-595.

71. Clark, W.G., Holdeman, M. and Lipton, J.M. (1985). Analysis of the antipyretic action of α-melanocyte stimulating hormone in rabbits. J. Physiol. 359: 459-465.

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

73. Coceani, F., Bishai, I., Dinarello, C.A. and Fitzpatrick, F.A. (1983). Prostaglandin $E_2$ and thromboxane $B_2$ in cerebrospinal fluid of afebrile and febrile cats. Am. J. Physiol. 244: R785-793.

74. Coley, W.B. (1919). Further observations on the conservative treatment of sarcoma of the long bones. Ann. Surg. 70: 633-660.

75. Cooper, K.E. (1987). The Neurobiology of Fever: Thoughts on Recent Developments. Ann. Rev. Neurosci. 10: 297-326.

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

77. 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.

78. Cooper, K.E., Cranston, W.I. and Snell, E.S. (1964). Temperature regulation during fever in man. Clin. Sci. 27: 345-356.

79. 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. 195: 33-45.

80. Cooper, K.E., Preston, E. and Veale, W.L. (1976). Effects of atropine injected into a lateral cerebral ventricle of the rabbit, on fevers due to intravenous leucocyte pyrogen and hypothalamic and intraventricular injections of prostaglandin $E_1$. J. Physiol. 254: 729-741.

81. 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.

82. Covert, J.B. and Reynolds, W.W. (1977). Survival value of fever in fish. Nature 267: 43-45.

83. 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. (Abstract)

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

85. 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.

86. Cranston, W.K., Hellon, R.F. and Mitchell, D. (1975). A dissociation between fever and prostaglandin concentration in cerebrospinal fluid. J. Physiol. 253: 583-592.

87. Cranston, W.I., Hellon, R.F., Mitchell, D. and Townsend, Y. (1983). Intraventricular injections of drugs which inhibit phospholipase $A_2$ suppress fever in rabbits. J. Physiol. 339: 97-105.

88. Cranston, W.I., Hellon, R.F. and Townsend, Y. (1980). Suppression of fever in rabbits by a protein synthesis inhibitor, anisomycin. J. Physiol. 305: 337-344.

89. 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.

90. Currie, J. (1798). Medical reports on the effects of water, cold and warm as a remedy in fever and other diseases, Vol. 1. Cadell and Davies, London (from Lomax 1979).

91. Cushing, H. (1931a) The reaction to posterior pituitary extract (pituitrin) when introduced into the cerebral ventricles. Proc. Natl. Acad. Sci. U.S.A. 17: 163-170.

92. Cushing, H. (1931b). The method of action of pituitrin introduced into the ventricle. Proc. Natl. Acad. Sci. U.S.A. 17: 239-247.

93. Damais, C., Riveau, G., Parant, M., Gerota, J. and Chedid, L. (1982). Production of lymphocyte activating factor in the absence of endogenous pyrogen by rabbit or human leukocytes stimulated by a muramyl dipeptide derivative. Int. J. Immunopharmacol. 4: 451-462.

94. Dascombe, M.J. (1983). Evidence against bradykinin as a mediator of fever in rabbits. In: Environment, Drugs and Thermoregulation (P. Lomax and E. Schönbaum, eds.) Karger, Basel pp. 141-143.

95. Dascombe, M.J. (1985). The Pharmacology of Fever. Prog. Neurobiol. 25: 327-373.

96. 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. Pharmacol. 66: 565-572.

97. Demotes-Mainard, J., Chauveau, J. Rodriquez, F., Vincent, J.O. and Poulain, D.A. (1986). Septal release of vasopressin in response to osmotic, hypovolemic, and electrical stimulation in rats. Brain Res. 381: 314-321.

98. Dempsey, R.A., Dinarello, C.A., Mier, J.W., Rosenwasser, L.J., Allegretta, M., Brown, T.E. and Parkinson, D.R. (1982). The differential effects of human leukocytic pyrogen/lymphocyte activating factor, T cell growth factor and interferon on human natural killer activity. J. Immunol. 129: 2504-2510.

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

100. De Vries, G.J., Buijs R.M., Van Leeuwen, F.W., Caffe, A.R. and Swaab, D.F. (1985). The vasopressinergic innervation of the brain in normal and castrated rats. J. Comp. Neurol. 233: 236-254.

101. DeWied, D. (1976). Behavioural effects of intracerebroventricularly administered vasopressin and vasopressin fragments. Life Sci. 19: 665-690.

102. DeWied, D., Gaffori, O., Van Ree, J.M. and De Jong, W. (1984). Vasopressin antagonists block peripheral as well as central vasopressin receptors. Pharm. Biochem. Behav. 21: 393-400.

103. Dinarello, C.A. (1979). Production of endogenous pyrogen. Fed. Proc. 38: 52-56.

104. Dinarello, C.A. (1984a). Interleukin-1. Rev. Infect. Dis. 6: 51-95.

105. Dinarello, C.A. (1984b). Interleukin-1: An important mediator of inflammation. Trends Pharm. Sci. 5: 420-422.

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

107. Dinarello, C.A., Clowes, G.H., Gordon, A.H., Saravis, C.A. and Wolff, S.M. (1984). Cleavage of human interleukin-1: Isolation of a peptide fragment from plasma of febrile humans and activated monocytes. J. Immunol. 133: 1332-1338.

108. Dinarello, C.A., Conti, P. and Mier, J.W. (1986). Effects of human interleukin-1 on natural killer cell activity: Is fever a host defence mechanism for tumor killing. Yale J. Biol. Med. 59: 97-106.

109. Dinarello, C.A., Chparber, M., Kent, E.F. and Wolff, S.M. (1981). Production of leukocytic pyrogen from phagocytes of neonates. J. Infect. Dis. 144: 337-343.

110. Dinarello, C.A., Marnoy, S.D. and Rosenwasser, L.J. (1983). Role of arachidonate metabolism in the immunoregulatory function of human leukocytic pyrogen/lymphocyte-activating factor/ interleukin-1. J. Immunol. 130: 890-895.

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

112. Dinarello, C.A. and Wolff, S.M. (1979). Mechanisms in the production of fever in humans. In: Seminars in Infectious Diseases Vol. 2 (L. Senstein and B.N. Fields, eds.) Atratton, New York pp. 173-192.

113. Dinarello, C.A. and Wolff, S.M. (1982). Molecular basis of fever in humans. Am. J. Med. 72: 799-819.

114. Disturnal, J.E. (1986). Electrophysiological studies on vasopressin mediated neurotransmission. M.Sc. Thesis, University of Calgary.

115. 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.

116. 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.

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

118. Dorsa, D.M., Majumdar, L.A. Petracca, F.M. Baskin, D.G. and Cornett, L.E. (1983). Characterization and localization of $^{3}H$-arginine$^{8}$-vasopressin binding to rat kidney and brain tissue. Peptides 4: 699-706.

119. DuBois, E.F. (1936). Basal metabolism in Health and Disease. Lea and Febiger, Philadelphia.

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

121. Duff, G.W. (1986). Is fever beneficial to the host: A clinical perspective. Yale J. Biol. Med. 59: 125-130.

122. Duff, B.W. and Durum, S.K. (1983). The pyrogenic and mitogenic actions of interleukin-1 are related. Nature 304: 449-451.

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

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

125. Eisenman, J.S. (1982). Electrophysiology of the anterior hypothalamus: Thermoregulation and fever. In: Pyretics and Antipyretics. Handbook of Experimental Pharmacology, Vol. 60, (A.S. Milton, ed.) Springer-Verlag, Berlin pp. 187-217.

126. Epstein, H.C., Hochwald, A. and Ashe, R. (1951). Salmonella infections of the newborn infant. J. Pediatr. 38: 723-731.

127. Eskay, R.L., Giraud, P., Oliver, C. and Brownstein, M.M. (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.

128. Feldberg, W. and Gupta, K.P. (1973). Pyrogen fever and prostaglandin-like activity in cerebrospinal fluid. J. Physiol. 228: 41-53.

129. Feldberg, W., Gupta, K.P., Milton, A.S. and Wendlandt, S. (1973). Effect of pyrogen and antipyretics on prostaglandin activity in cisternal CSF of anaesthetised cats. J. Physiol. 234: 279-303.

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

131. Ferris, C.F., Albers, H.E., Weslowski, S.M., Goldman, B.D. and Luman, S.E. (1984). Vasopressin injected into the hypothalamus triggers a stereotypic behaviour in Golden hamsters. Science 224: 521-523.

132. Fessler, J.H., Cooper, K.E., Cranston, W.I. and Vollum, R.L. (1961). Observations on the production of pyrogenic substances by rabbits and human leucocytes. J. Exp. Med. 113: 1127-1140.

133. Fibiger, H.C. (1982). The organization and some projections of cholinergic neurons of the mammalian forebrain. Brain Res. Rev. 4: 327-388.

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

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

136. 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)

137. Fulton, J.F. and Wilson, L.G. (1966). Selected Readings in the History of Physiology. C.C. Thomas, Springfield.

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

139. Gander, G.W. and Goodale, F. (1975). The role of granulocytes and mononuclear leukocytes in fever. In: Temperature Regulation and Drug Action (P. Lomax, E. Schönbaum and J. Jacob, eds.) Karger, Basel pp. 51-58.

140. Gerbandy, J., Cranston, W.I. and Snell, E.S. (1954). The initial process in the actions of bacterial pyrogens in man. Clin. Sci. 13: 453-459.

141. Gery, I. Gershon, R.K. and Waksman, B.H. (1972). Potentiation of the T-lymphocyte response to mitogens. I. The responding cell. J. Exp. Med. 136: 128-142.

142. Gilman, S.C., Rosenberg, J.S. and Feldman, J.D. (1983). Inhibition of interleukin-1 synthesis and T cell proliferation by a monoclonal anti Ia antibody. J. Immunol. 130: 1236-1240.

143. 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.

144. 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.

145. Goldstein, I.R., Kaplan, H.B., Edelson, H.S. and Weissman, G. (1982). Ceruloplasmin: An acute phase reactant that scavenges oxygen-derived free radical. Ann. N.Y. Acad. Sci. 389: 378-383.

146. Gordon, C.J. and Heath, J.E. (1979). The effect of prostaglandin $E_2$ on the firing rate of thermally sensitive and insensitive neurons in the preoptic/anterior hypothalamus of unanesthetized rabbits. Fed. Proc. 38: 1295. (Abstract)

147. Grant, R. and Whalen, W.J. (1953). Latency of pyrogen fever. Appearance of a fast-acting pyrogen in the blood of febrile animals and in plasma incubated with bacterial pyrogen. Am. J. Physiol. 173: 47-54.

148. Grollman, A.P. and Walsh, M. (1967). Inhibitors of protein biosynthesis. II. Mode of action of anisomycin. J. Biol. Chem. 242: 3226-3233.

149. Grundmann, M.J. (1969). Studies on the action of antipyretic substances. D. Phil. Thesis, University of Oxford.

150. Hales, J.R.S., Bennett, 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 Archiv. 339: 125-133.

151. Hampton, G.R., Sharp, W.V. and Andresen, G.J. (1973). Long-term rabbit restraint - a simple method. Lab. Anim. Sci. 23: 590-591.

152. Hellon, R.F. and Townsend, Y. (1983). Mechanisms of fever. Pharmac. Ther. 19: 211-244.

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

154. 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.

155. 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.

156. Horita, A. and Corino, M.A. (1975). Thyrotropin releasing hormone (TRH)-induced hyperthermia and behavioural excitation in rabbits. Psychopharmac. Commun. 1: 403-414.

157. Hunter, J. (1778). Of the heat of animals and vegetables. Phil. Trans. 68: 7-49. (from Fulton and Wilson, 1966).

158. Isenschmid, R. and Schnitzler, W. (1914). Beitrag zur Lokalisation des der Wärmeregulation vorstehenden Zentralapparates im Zwischenhirn. Arch. Exp. Pathol. Pharmakol. 76: 202-223. (from Lomax, 1979).

159. Ishikawa, S., Saito, T. and Yoshida, S. (1981). The effects of prostaglandins on the release of arginine vasopressin from the guinea-pig hypothalamo-neurohypophyseal complex in organ culture. Endocrinology 108: 192-198.

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

161. Jasper, H.H. and Ajmone-Marsan, C. (1954). A Stereotaxic Atlas of the Diencephalon of the Cat. National Research Council, Ottawa, Canada.

162. Jell, R.M. and Sweatman, P. (1977). Prostaglandin sensitive neurons in cat hypothalamus: relation to thermoregulation and to biogenic amines. Can. J. Physiol. Pharmacol. 54: 161-166.

163. Joels, M. and Urban, I.J.A. (1982). The effect of microiontophoretically applied vasopressin and oxytocin on single neurons in the septum and hippocampus of the rat. Neurosci. Lett. 33: 79-84.

164. Joels, M. and Urban, I.J.A. (1984). Arginine$^{8}$-vasopressin enhances the responses of lateral septal neurons in the rat to excitatory amino acids and fimbria-fornix stimulation. Brain Res. 311: 201-209.

165. Jonasson, H., Basu, S., Andersson, B. and Kindahl, H. (1984). Renal excretion of prostaglandin metabolites, arginine vasopressin and sodium during endotoxin and endogenous pyrogen induced fever in the goat. Acta Physiol. Scand. 120: 529-536.

166. Jones, C.T. Boddy, K. and Robinson, J.S. (1977). Changes in the concentration of adrenocorticotrophin and corticosteroid in the plasma of the fetal lamb in the latter half of pregnancy and during labour. J. Endocrinol. 72: 293-300.

167. Kampschmidt, R.F. (1981). Leukocytic endogenous mediator/endogenous pyrogen. In: The Physiologic and Metabolic Responses of the Host (M.C. Powanda and P.G. Canonico, eds.) Elsevier North Holland, Amsterdam pp. 55-74.

168. Kampschmidt, R.F. (1984). Infection, inflammation and interleukin-1. Lymphokine Res. 2: 97-110.

169. Kampschmidt, R.F., Upchurch, H.F., Eddington, D.L. and Pulliam, L.A. (1973). Multiple biological activities of a partially purified leukocytic endogenous mediator. Am. J. Physiol. 224: 530-533.

170. Kandasamy, S.B. and Williams, B.A. (1983a). Absence of endotoxin fever but not hyperthermia in Brattleboro rats. Experientia 39: 1343.

171. Kandasamy, S.B. and Williams, B.A. (1983b). Cholecystokinin octapeptide-induced hyperthermia in guinea-pigs. Experientia 39: 1282-1284.

172. Kandasamy, S.B. and Williams, B.A. (1983c). Hyperthermic responses to central injections of some peptide and non-peptide opioids in the guinea-pig. Neuropharmacology 22: 621-628.

173. Kandasamy, S.B. and Williams, B.A. (1984). Hypothermic and antipyretic effects of ACTH 1-24 and α-melanotropin. Neuropharmacology 23: 49-53.

174. Kasting, N.W. (1980). An antipyretic system in the brain and the role of vasopressin. Ph.D. Thesis, University of Calgary.

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

176. 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.

177. Kasting, N.W., Carr, D.B., Martin, J.B., Blume, H. and Bergland, R. (1983). Changes in cerebrospinal fluid and plasma vasopressin in the febrile sheep. Can. J. Physiol. Pharmacol. 61: 427-431.

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

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

180. 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.

181. 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.

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

183. Kasting, N.W., Veale, W.L., Cooper, K.E. and Lederis, K. (1979c). An endogenous antipyretic produced in late pregnancy. In: Thermoregulatory Mechanisms and Their Therapeutic Implications (B. Cox, P. Lomax, A.S. Milton, E. Schönbaum, eds.) Karger, Basel, pp. 95-99.

184. Kasting, N.W., Veale, W.L., Cooper, K.E. and Lederis, K. (1981). Effect of hemorrhage on fever: The putative role of vasopressin. Can. J. Physiol. Pharmacol. 59: 324-328.

185. Kasting, N.W., Veale, W.L. and Cooper, K.E. (1982). Vasopressin: A homeostatic effector in the febrile process. Neurosci. Biobehav. Rev. 6: 215-222.

186. 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.

187. Kenedi, E., Laburn, H., Mitchell, D. and Ross, F.P. (1982). On the pyrogenic action of intravenous lipid A in rabbits. J. Physiol. 328: 361-370.

188. Kirk, C.J., Rodrigues, 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.

189. 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.

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

191. Kluger, M.J. (1986). Is fever beneficial? Yale J. Biol. Med. 59: 89-95.

192. Kluger, M.J., Ringler, D.H. and Anver, M.R. (1975). Fever and survival. Science 188: 166-168.

193. Kluger, M.J. and Rothenberg, B.A. (1979). Fever and reduced iron: Their interaction as a host defence response to bacterial infection. Science 203: 374-376.

194. Kluger, M.J. and Vaughn, L.K. (1978). Fever and survival in rabbits infected with Pasteurella multocida. J. Physiol. 282: 243-251.

195. Knudsen, P.J., Dinarello, C.A. and Strom, T.B. (1986). Purification and characterization of a unique human interleukin-1 from the tumor cell line U937. J. Immunol. 136: 3311-3316.

196. Koretzky, G.A., Daniele, R.P., Greene, W.C. and Nowell, P.C. (1983). Evidence for an interleukin-independent pathway for human lymphocyte activation. Proc. Natl. Acad. Sci. U.S.A. 80: 3444-3447.

197. Kovacs, G.L. and DeWied, D. (1983). Hormonally active vasopressin suppresses endotoxin-induced fever in rats. Lack of effect of oxytocin and a behaviorally active vasopresin fragment. Neuroendocrinology 37: 258-261.

198. Kreuger, J.M., Walter, J., Dinarello, C.A., Wolff, S.M. and Chedid, L. (1984). Sleep-promoting effects of endogenous pyrogen (interleukin-1). Am. J. Physiol. 246: R994-R999.

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

200. 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.

201. Kruszynski, M., Lammek, B., Manning, M., Seto, J., Haldar, J. and Sawyer, W.H. (1980). [1-(β-mercapto-β,β-cyclopentamethylene-propionic 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.

202. Kunkel, S.L., Chensue, S.W. and Phan, S.H. (1986). Prostaglandins as endogenous mediators of interleukin-1 production. J. Immunol. 136: 186-192.

203. Kushner, I. and Kaplan, M.H. (1961). Studies of acute phase protein. I. An immunohistochemical method for the localization of Cx-reactive protein in rabbits. Association with necrosis in local inflammatory lesions. J. Exp. Med. 114: 961-974.

204. Laburn, H.P., Mitchell, D., Kenedi, E. and Louw, G.N. (1981). Pyrogens fail to produce fever in cordylid lizard. Am. J. Physiol. 241: R198-R202.

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

206. Laburn, H., Mitchell, D. and Stephen, J. (1980). Effects of intracerebroventricular floctaferine and indomethacin on body temperatures in febrile rabbits. Br. J. Pharmacol. 71: 525-528.

207. Lachman, L.B. (1983). Interleukin-1; release from LPS-stimulated mononuclear phagocytes. In: Beneficial effects of endotoxin (A. Nowotny, ed.) Plenum Press, New York pp. 283-305.

208. Land, H., Schultz, G., Schmale, H. and Richter, D. (1982). Nucleotide sequence of cloned cDNA encoding bovine arginine-vasopressin-neurophysin II precursor. Nature 295: 299-303.

209. Lavoisier, A.-L. (1777). "Experiences sur la respiration des animaux, et sur les changements qui arrivent a l'air en passant par leeur poumon. Mem. Hist. Acad. roy. Sci. Paris, 1780, pp. 185-194. (from Fulton and Wilson, 1966).

210. Lee, T.F., Mora, F. and Myers, R.D. (1985). Effect of intracerebroventricular vasopressin on body temperature and endotoxin fever of macaque monkey. Am. J. Physiol. 248: R674-R679.

211. Li, D.J., Hahn, G.M. (1984). Responses of RIF tumors to heat and drugs: Dependence on tumor size. Cancer Treat. Rep. 68: 1149-1151.

212. Liao, Z., Grimshaw, R.S. and Rosenstretch, D.L. (1984). Identification of a specific interleukin-1 inhibitor in the urine of febrile patients. J. Exp. Med. 159: 126-136.

213. Liebermeister, C. (1871). Ueber Wärme-Regulierung und Fieber. Heft 19, Sammlung Klinischer Vorträge in Verbindung mit deutschen Klinikern, herausgegeben von Richard Volkmann, Breitkopf und Härtel, Leipzig.

214. Liebermeister, C. (1887). Vorlesungen über spezielle Pathologie und Therapie. (Verlag von F.C.W. Vogel, Leipzig).

215. Liggins, G.C., Fairclough, R.J., Grieves, S.A., Kendall, J.Z. and Knox, B.S. (1973). The mechanism of initiation of parturition in the ewe. Rec. Prog. Horm. Res. 29: 111-150.

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

217. Lin, M.T., Wang, T.I., and Chan, H.K. (1983). A prostaglandin-adrenergic link occurs in the hypothalamic pathways which mediate the fever induced by vasopressin in the rat. J. Neural. Trans. 56: 21-31.

218. Lipsky, P.E., Thompson, P.A., Rosenwasser, L.J. and Dinarello, C.A. (1983). The role of interleukin-1 in human B cell activation: inhibition of B cell proliferation and the generation of immunoglobulin secreting cells by an antibody against human leukocytic pyrogen. J. Immunol. 130: 2708-2714.

219. Lipton, J.M. and Clark, W.G. (1986). Neurotransmitters in temperature control. Ann. Rev. Physiol. 48: 613-623.

220. Lipton, J.M. and Glyn, J.R. (1980). Central administration of peptides alters thermoregulation in the rabbit. Peptides 1: 15-18.

221. Lipton, J.M. and Kennedy, J.I. (1979). Central thermosensitivity during fever produced by intra PO/AH and intravenous injection of pyrogen. Brain Res. Bull. 4: 23-34.

222. Lipton, J.M. and Trzcinka, G.P. (1976). Persistance of febrile response to pyrogens after PO/AH lesions in squirrel monkeys. Am. J. Physiol. 231: 1638-1648.

223. Lomax, E. (1979). Historical development of concepts of thermoregulation. In: Body Temperature (P. Lomax, E. Schönbaum, eds.) Dekker, New York pp 1-23.

224. Lomedico, P.T. Gubler, V., Hellmann, C.P. Dukovich, M., Giri, G. Pan, Y-C.E., Collier, K., Seminow, R., Chua, A.O. and Mizel, S.B. (1984). Cloning and expression of murine interleukin-1 cDNA in Escherichia coli. Nature 312: 458-462.

225. Lu, C.L., Cantin, M., Seidah, N.G. and Chretien, M. (1982). Immunohistochemical localization of human pituitary glycopeptide (HPGP)-like immunoreactivity in the hypothalamus and pituitary of normal and homozygous Diabetes insipidus (Brattleboro) rats. J. Histochem. Cytochem. 30: 999-1003.

226. 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.

227. Lüderitz, 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.

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

229. 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.

230. Manning, M., Klis, W.A., Olma, A., Seto, J. and Sawyer, W.H. (1982). Design of more potent and selective antagonists of the antidiuretic responses to arginine-vasopressin devoid of antidiuretic agonism. J. Med. Chem. 25: 414-419.

231. Marchand, J.E. and Hagino, N. (1982). Effect of iontophoresis of AVP on lateral septal neurons. Exp. Neurol. 78: 790-795.

232. Marx, J., Hilbig, R. and Rahmann, H. (1984). Endotoxin and prostaglandin $E_1$ fail to induce fever in a teleost fish. Comp. Biochem. Physiol. 77a: 483-487.

233. McCarthy, D.M., Kluger, M.J. and Vander, A.J. (1985). Suppression of food intake following endotoxin: Is interleukin-1 involved? In: Physiologic, Metabolic and Immunolgic Actions of Interleukin-1 (M.J. Kluger, J.J. Oppenheim, M.C. Powanda, eds.) A.R. Liss, New York pp. 171-179.

234. Meisenberg, G. and Simmons, W.H. (1984a). Hypothermia induced by centrally administered vasopressin in rats. A structure-activity study. Neuropharmacology 23: 1195-1200.

235. Meisenberg, G. and Simmons, W.J. (1984b). Factors involved in the inactivation of vasopressin after intracerebroventricular injection in mice. Life Sci. 34: 1231-1240.

236. Merker, G., Blähser, S. and Zeisberger, E. (1980). The reactivity pattern of vasopressin containing neurons and its relations to the antipyretic reaction of the guinea pig. Cell Tiss. Res. 212: 47-61.

237. Merriman, C.R., Pulliam, L.A. and Kampschmidt, R.F. (1977). Comparison of leukocytic pyrogen and leukocytic endogenous mediator. Proc. Soc. Exp. Biol. Med. 154: 224-227.

238. Miller, T.R. and Nicholson, J.T. (1971). End results in reticulum cell sarcoma of bone treated by bacterial toxin therapy alone or combined with surgery and/or radiotherapy (47 cases) or with concurrent infection (5 cases). Cancer 27: 524-548.

239. 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, Berlin pp. 257-303.

240. Milton, A.S. and Sawhney, V.K. (1981). The effects of anisomycin on the febrile responses to intracerebroventricular bacterial pyrogen and prostaglandin $E_2$ in cats. Br. J. Pharmacol. 74: 786P. (Abstract)

241. Milton, A.S. and Sawhney, V.K. (1982). Protein Synthesis and Fever. In: Pyretics and Antipyretics. Handbook of Experimental Pharmacology, Vol. 60 (A.S. Milton, ed.) Springer-Verlag, Berlin pp. 305-315.

242. Milton, A.S. and Wendlandt, S. (1970). A possible role for prostaglandin $E_1$ as a modulator for temperature regulation in the central nervous system of the cat. J. Physiol. 207: 76-77P (Abstract).

243. 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.

244. 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.

245. Mitchell, D., Laburn, H.P., Cooper, K.E., Hellon, R.F., Cranston, W.I. and Townsend, Y. (1986). Is prostaglandin E the neural mediator of the febrile response? The case against a proven obligatory role. Yale J. Biol. Med. 59: 159-168.

246. Mitchell, D., Snellen, J.W. and Atkins, A.R. (1970). Thermoregulation during fever: Change of set point or change of gain. Pflügers Arch. Gest. Physiol. 321: 293-302.

247. Mizel, S.G. (1982). Interleukin-1 and T cell activation. Immunol. Rev. 63: 51-72.

248. Mizuno, Y. Oomura, Y., Hori, N. and Carpenter, D.O. (1984). Action of vasopressin on $CA_1$ pyramidal neurons in rat hippocampal slice. Brain Res. 309: 241-246.

249. Mold, C., Duclos, T.W., Nakayama, S., Edwards, K.M. and Gewurz, H. (1982). C-reactive protein reactivity with complement and effects on phagocytosis. Ann. N.Y. Acad. Sci. 389: 251-259.

250. Mühlethaler, M., Dreifuss, J.J. and Gahwiler, B.H. (1982). Vasopressin excites hippocampal neurons. Nature 296: 749-751.

251. Mühlethaler, M., Sawyer, W.H., Manning, M.M. and Dreifus, J.J. (1983). Characterization of a uterine type oxytocin receptor in the rat hippocampus. Proc. Natl. Acad. Sci. U.S.A. 80: 6713-6717.

252. 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 Tiss. Res. 219: 85-92.

253. Myers, R.D. (1971). Methods for chemical stimulation of the brain. In: Methods in Psychobiology, Vol. 1 (R.D. Myers, ed.) Academic Press, New York, pp. 247-280.

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

255. Myers, R.D. and Veale, W.L. (1970). Body temperature: Possible ionic mechanisms in the hypothalamus controlling the set point. Science 170: 95-97.

256. 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.

257. Naunyn, B. and Quincke, H. (1869). Über den Einfluss des Zentralnervensystems auf die Wärmebildung im Organismus. Arch. Anat. Physiol. Wissenschaft Med. 174-199, 521-533. (from Lomax 1979).

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

259. Oppenheim, J. (1986). Interleukin-1. Immunology Today 7: 186 (news and features).

260. Oppenheim, J.J., Stadler, B.M., Siraganian, R.P., Mage, M. and Mathieson, B. (1982). Lymphokines: Their role in lymphocyte responses. Properties of interleukin-1. Fed. Proc. 41: 257-262.

261. Ott, I. (1884). The relation of the nervous system to the temperature of the body. J. Nerv. Ment. Dis. 11: 141-152. (from Lomax, 1979).

262. Parant, M., Riveau, G., Parant, F., Dinarello, C.A., Wolff, S.M. and Chedid, L. (1980). Effect of indomethacin on increased resistance to bacterial infection and on febrile responses induced by muramyl dipeptide. J. Infect. Dis. 142: 708-715.

263. Paxinos, G. and Watson, C. (1982). The Rat Brain in Stereotaxic Coordinates. Academic Press, Sydney.

264. Pearlmutter, A.F., Costantini, M.G. and Loeser, B. (1983). Characterization of $^{3}$H-AVP binding sites in particulate preparations of rat brain. Peptides 4: 335-341.

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

266. Pickering, B.T., Swann, R.W. and Gonzalez, C.B. (1983). Biosynthesis and processing of neurohypophyseal hormones. Pharmac. Ther. 22: 143-161.

267. Pittman, Q.J., Blume, H.W. and Renaud, L.P. (1981). Connections of the hypothalamic PVN with neurohypophysis, median eminence, amygdala, lateral septum and midbrain periaqueductal gray: An electrophysiological study in the rat. Brain Res. 215: 15-25.

268. Pittman, Q.J., Cooper, K.E., Veale, W.L. and Van Petten, G.R. (1974). Observations on the development of the febrile response to pyrogens in the sheep. Clin. Sci. Mol. Med. 46: 581-592.

269. Pittman, Q.J. and Franklin, L.G. (1985). Vasopressin antagonist in nucleus tractus solitarius/vagal area reduces pressor and tachycardia responses to paraventricular nucleus stimulation in rats. Neurosci. Letters. 56: 155-160.

270. Pittman, Q.J., Riphagen, C.L., and Lederis, K. (1984). Release of immunoassayable neurohypophyseal peptides from rat spinal cord, in vivo. Brain Res. 300: 321-326.

271. Pittman, Q.J., Veale, W.L. and Cooper, K.E. (1977). Absence of fever following intrahypothalamic injections of prostaglandins in sheep. Neuropharmacology 16: 743-749.

272. Pittman, Q.J., Veale, W.L. and Lederis, K. (1982). Central neurohypophyseal peptide pathways - interactions with endocrine and other autonomic functions. Peptides 3: 515-520.

273. Porter, J.P. and Brody, M.J. (1986). A $V_1$ vasopressin receptor antagonist has nonspecific neurodepressant actions in the spinal cord. Neuroendocrinology 43: 75-78.

274. Prohaska, J.R. and Lukasewycz, O.A. (1981). Copper deficiency suppresses the immune response of mice. Science 213: 559-561.

275. 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.

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

277. Richet, C. (1885). Die Beziehungen des Gehirns zur Körperwärme und zum Fieber. Pflügers Arch. 37: 624-625. (from Lomax 1979).

278. Richet, C. (1898). Chaleur. Dictionnaire de Physiologie Vol. 3 Baillière, Paris pp. 81-271. (from Lomax 1979).

279. Rietschel, E.T., Kim, Y.B., Watson, D.W., Galanos, C., Lüderitz, O. and Westphal, O. (1973). Pyrogenicity and immunogenicity of lipid A complexed with bovine serum albumin. Infect. Immun. 8: 173-177.

280. Rimsky, L., Wakasugi, H., Ferrara, P., Robin, P., Capdevielle, J., Tursz, T., Fradelizi, D. and Bertoglio, J. (1986). Purification to homogeneity and $NH_2$-terminal amino acid sequence of a novel interleukin-1 species derived from a human B cell line. J. Immunol. 136: 3304-3310.

281. Rosenwasser, L.J. and Dinarello, C.A. (1981). Ability of human leukocytic pyrogen to enhance phytohemagglutinin immune thymocyte proliferation. Cell Immunol. 63: 134-142.

282. 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.

283. Rudy, T.A., Williams, J.W. and Yaksh, T.L. (1977). Antagonism by indomethacin of neurogenic hyperthermia produced by unilateral puncture of the anterior hypothalamic/preoptic region. J. Physiol. 272: 721-736.

284. Ruwe, W.D. and Myers, R.D. (1979). Fever produced by intrahypothalamic pyrogen: Effect of protein synthesis inhibition by anisomycin. Brain Res. Bull. 4: 741-745.

285. 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.

286. Ruwe, W.D., Veale, W.L. and Pittman, Q.J. (1986). Electrical stimulation of a discrete site in the brain suppresses pyrogen fever in the rabbit. Proceedings Satellite Symposium on Thermal Physiology 55 (Abstract).

287. Ruwe, W.D., Veale, W.L., Pittman, Q.J., Kasting, N.W., and Lederis, K. (1985). 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 pp. 233-247.

288. 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.

289. Satinoff, E., McEwen, G.N. and Williams, B.A. (1976). Behavioral fever in newborn rabbits. Science 193: 1139-1140.

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

291. 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.

292. Sawyer, W.H., Acosta, M. and Manning, M. (1974). Structural changes in the arginine vasopressin molecule that prolong its antidiuretic action. Endocrinology 95: 140-149.

293. Sawyer, C.H., Everett, J.W. and Green, J.D. (1954). The rabbit diencephalon in stereotaxic coordinates. J. Comp. Neurol. 101: 801-824.

294. Schmale, H., Heinsohn, S. and Richter, D. (1983). Structural organization of the rat gene for the arginine-vasopressin-neurophysin precursor. EMBO J. 2: 763-767.

295. Schoener, E.P. and Wang, S.C. (1975). Leucocyte pyrogen and sodium acetylsalicylate on hypothalamic neurons in the cat. Am. J. Physiol. 229: 185-190.

296. Schoener, E.P. and Wang, S.C. (1976). Effects of locally administered prostaglandin $E_1$ on anterior hypothalamic neurons. Brain Res. 117: 157-162.

297. Schwartzkroin, P.A. (1975). Characteristics of CA 1 neurons recorded intracellularly in the hippocampal *in vitro* slice preparation. Brain Res. 85: 423-436.

298. Seidah, N.G., Benjannet, S. and Chretien, M. (1981). The complete sequence of a novel human pituitary glycopeptide homologous to pig posterior pituitary glycopeptide. Biochem. Biophys. Res. Commun. 100: 901-907.

299. Sheth, U.K. and Borison, H.L. (1960). Central pyrogenic action of *Salmonella typhosa* lipopolysaccharide injected into the lateral cerebral ventricle in cats. J. Pharm. Exp. Ther. 130: 411-417.

300. 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: R803-R806.

301. Siegert, R., Philipp-Dormstrom, W.K., Radsak, K. and Menzel, H. (1976). Mechanism of fever induction in rabbits. Infect. Immun. 14: 1130-1137.

302. Sigal, S.L. (1978). Fever theory in the seventeenth century: Building toward a comprehensive physiology. Yale J. Biol. Med. 51: 571-582.

303. Sinding, C., Camier, M. and Cohen, P. (1982). Extra-hypothalamo-neurohypophyseal immunoreactive neurophysin occurs predominantly as high M[r] forms in the rat brain stem. FEBS Lett. 140: 124-126.

304. Smith, K.A., Gilbridge, K.J. and Favata, M.F. (1980). Lymphocyte activating factor promotes T cell growth factor production by cloned murine lymphoma cells. Nature 287: 353-354.

305. Smith, R.T., Platou, E.S. and Good, R.A. (1956). Septicemia of the newborn. Pediatrics 17: 549-575.

306. Sofroniew, M.V. (1983). 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, Amsterdam pp. 101-114.

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

308. Stein, M., Schiari, R.C. and Camerino, M. (1976). Influence of brain and behavior on the immune system. Science 191: 435-440.

309. Stitt, J.T. (1973). Prostaglandin $E_1$ induced fever in rabbits. J. Physiol. 232: 163-179.

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

311. 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, E. Schönbaum, eds.) Karger, Basel pp. 120-125.

312. 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.

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

314. 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.

315. Stitt, J.T. and Hardy, J.D. (1975). Microelectrophoresis of $PGE_1$ onto single units in rabbit hypothalamus. Am. J. Physiol. 229: 240-245.

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

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

318. Sugarman, B. (1983). Zinc and infection. Rev. Infect. Dis. 5: 137-147.

319. 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.

320. 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. (Abstract)

321. Tindal, J.S. (1965). The forebrain of the guinea-pig in stereotaxic coordinates. J. Comp. Neurol. 124: 259-266.

322. Townsend, Y., Cranston, W.I. and Hellon, R.F. (1984). Inhibition of brain protein synthesis suppresses the release of prostaglandin $E_2$ in febrile rabbits. Brain Res. Bull. 13: 335-338.

323. 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.

324. Tscheschichin, J. (1866). Zur Lehre von der thierischen Wärme. Arch. Anat. Physiol. pp. 151-179. (from Lomax 1979).

325. Valtin, H., Sawyer, W.H. and Sokol, H.W. (1965). Neurohypophyseal principles in rats homozygous and heterozygous for hypothalamic diabetes insipidus (Brattleboro strain). Endocrinology 77: 701-706.

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

327. Van Leeuwen, F. and Caffe, R. (1983). Vasopressin-immunoreactive cell bodies in the bed nucleus of the stria terminalis of the rat. Cell Tiss. Res. 228: 525-534.

328. Van Miert, A.S.J.A.M., Van Essen, J.A. and Tromp, G.A. (1971). The antipyretic effect of pyrozalone derivative and salicylates on fever induced with leukocytes or bacterial pyrogen. Arch. Int. Pharmacodyn 197: 288-391.

329. Vaughn, L.K., Bernheim, M.A. and Kluger, M.J. (1974). Fever in the lizard Dipsosaurus dorsalis. Nature 252: 473-474.

330. Veale, W.L. (1971). Behavioral and physiological changes caused by the regional alteration of sodium and calcium ions in the hypothalamus of the unanesthetized cat. Thesis, Purdue University.

331. 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.

332. Veale, W.L., Kasting, N.W. and Cooper, K.E. (1981). Arginine vasopressin and endogenous antipyresis: Evidence and significance. Fed. Proc. 40: 2750-2753.

333. Vilhardt, J. and Hedquist, P. (1970). A possible role for prostaglandin $E_2$ in the regulation of vasopressin secretion in rats. Life Sci. 9: 825-830.

334. Villablanca, J. and Myers, R.D. (1965). Fever produced by microinjection of typhoid vaccine into hypothalamus of cats. Am. J. Physiol. 208: 703-706.

335. Wannemacher, R.W. (1977). Key role of various individual amino acids in the host response to infection. Am. J. Clin. Nutr. 30: 1269-1280.

336. Weinberg, E.D. (1978). Iron and infection. Microbiol. Rev. 42: 45-66.

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

338. Weiss, B., Laties, V.G. and Weiss, A.B. (1967). Behavioral thermoregulation by cats with pyrogen-induced fever. Archs. Int. Pharmacodyn. Ther. 165: 467-475.

339. Welch, W.H. (1888). The Cartwright Lectures on the general pathology of fever. Med. News (Philadelphia) 52: 365, 393, 539, 565. (from Lomax 1979).

340. 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).

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

342. 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.

343. 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 interleukin-1 all exhibit similar biologic activities. J. Immunol. 134: 895-903.

344. Wunderlich, C.A. (1871). On the temperature in disease: A manual of medical thermometry. New Sydenham Society, London.

345. Yamamoto, M., Share, L. and Shade, R.E. (1976). Vasopressin release during ventriculocisternal perfusion with prostaglandin $E_2$ in the dog. J. Endocrinol. 71: 325-331.

346. Yamamura, H.I., Gee, K.W., Brinton, R.E., Davis, T.P., MacHadley, D. and Wamsley, J.K. (1983). Light microscopic autoradiographic visualization of [$^3$H]-arginine vasopressin binding sites in rat brain. Life Sci. 32: 1919-1924.

347. Zeisberger, E., Merker, G. and Blähser, S. (1981). Fever response in the guinea-pig before and after parturition. Brain Res. 212: 379-392.

348. Zeisberger, E., Merker, S., Blähser, S. and Kranning, M. (1986). Role of vasopressin in fever regulation. In: Pharmacology of Thermoregulation Homeostasis and Thermal Stress: Experimental and Therapeutic Advances. (K.E. Cooper, P. Lomax, E. Schönbaum and W.L. Veale, eds.) Karger, Basel pp. 62-65.

349. Ziel, R., Krupp, P. (1975). Effect on prostaglandin synthesis and antipyretic activity of non steroid antiinflammatory drugs. In: Temperature Regulation and Drug Action (P. Lomax, E. Schönbaum and J. Jacob, eds.) Karger, Basel pp. 233-241.

350. Zimmerman, E.A., Robinson, A.G., Husain, M.K., Acosta, M., Franz, A.G. and Sawyer, W.H. (1974). Neurohypophysial peptides in the bovine hypothalamus: the relationship of neurophysin I to oxytocin and neurophysin II to vasopressin in supraoptic and paraventricular regions. Endocrinology 95: 931-937.