AN ANALYSIS OF THE ANTIPYRETIC EFFECTS OF CENTRALLY ADMINISTERED ARGININE VASOPRESSIN IN THE RAT

By

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ABSTRACT

Previous studies in the sheep, rabbit, cat and rat have demonstrated the ability of the neuropeptide, arginine vasopressin (AVP), to suppress endotoxin-induced fever when perfused into a discrete brain locus. Fever can also be suppressed if AVP is microinjected into the cerebral ventricles of the rat. The mechanisms by which AVP mediates antipyresis are unknown. Experiments were conducted, therefore, to examine the effect of intracerebroventricular (icv) AVP on an established fever and to assess the mechanism of action using a specific, V_1-receptor antagonist (M-AVP). Studies were also conducted to elucidate the effector mechanisms utilized to accomplish antipyresis induced by icv AVP. Finally, cerebrospinal fluid (CSF) AVP concentrations were measured in febrile and non-febrile rats to determine the role of endogenously released AVP in the CSF during fever.

AVP administered icv was shown to have marked antipyretic effects at very low doses. This antipyresis was elicited in rats with an established fever but the peptide had no effect on the temperature of non-febrile rats. Thus AVP both prevented and reversed endotoxin-induced fever. Furthermore, this AVP-induced antipyresis was abolished by pretreatment with the the V_1-antagonist, M-AVP. The antipyretic effects of AVP were, therefore, receptor mediated and likely to be of physiological importance.

Efforts to manipulate the endogenous AVP system by icv M-AVP were also attempted. When M-AVP was injected icv, the fever height of endotoxin-treated rats was not different from endotoxin-treated controls. In addition, M-AVP did not influence the magnitude of the antipyresis induced by indomethacin. It has become clear, however, that this method of administering the antagonist is inappropriate to block endogenous AVP

effects occurring within the neuropil. Subsequent experiments in another laboratory have shown that M-AVP must be microinjected into the AVP-sensitive brain locus to effectively block endogenous activity.

The antipyretic response to icv AVP was further investigated at three ambient temperatures in an attempt to identify the effector mechanisms involved. Responses of non-febrile and febrile rats to icv injections of AVP and sc injections of indomethacin were observed at cold (4^{0}C), neutral (25^{0}C) and warm (32^{0}C) ambient temperatures. As in the previous experiments, AVP at 25^{0}C decreased brain temperatures of febrile but not non-febrile rats. This antipyretic effect was also observed at the warm ambient temperature and during cold exposure. Responses to sc indomethacin were qualitatively similar to icv AVP at neutral and warm temperatures. In the cold, however, indomethacin decreased the brain temperature of both non-febrile and febrile animals, although unlike AVP, brain temperature of non-febrile animals decreased somewhat more than that of febrile animals. These data showed that AVP decreased brain temperature of febrile more so than non-febrile rats at all ambient temperatures and may therefore have been acting partially on febrile set-point. It was possible that AVP affected specific effector mechanisms since antipyretic effects were of different magnitudes at different ambient temperatures. The observation that AVP and indomethacin had qualitatively similar effects on fever at three ambient temperatures suggested that they may act via a common neural pathway.

Further analysis of the mechanism of icv AVP-induced antipyresis was conducted at the three ambient temperatures while measuring specific effectors: heat loss and heat production. At 25^{0}C, AVP-induced antipyresis was mediated by tail skin vasodilation while metabolic rate was unaffected. At 4^{0}C, the antipyresis produced by AVP was mediated exclusively by inhibition of heat production since the metabolic rate decreased markedly following AVP. This antipyresis at 4^{0}C was accompanied by cutaneous vasoconstriction.

At 32°C, neither vasomotor tone, metabolic rate nor evaporative heat loss could be shown to contribute to the small antipyretic effect elicited by AVP. These data strongly suggest that icv AVP produced antipyresis by affecting the febrile body temperature set-point mechanism since the thermoregulatory strategy to lose heat varied at different ambient temperatures and the decrease in body temperature could not be shown to be due to changes in a single effector mechanism.

As an index of endogenous AVP activity, cerebrospinal fluid (CSF) concentrations of AVP were measured in febrile and non-febrile rats in order to determine the role of CSF AVP in fever and antipyresis. The results demonstrated that the AVP release pattern was not altered in endotoxin-treated febrile compared to non-febrile rats. It was concluded that CSF AVP had no role in the febrile process.

In summary, icv AVP appears to induce antipyresis by its action on febrile set-point rather than on a specific effector system. This action of AVP is mediated by a V_1-like receptor mechanism which is not affected by endogenous CSF AVP. The neural/neurochemical basis for the thermoregulatory set-point has not been clearly established so the mechanism of action by which AVP affects set-point remains to be determined. These data contribute, however, to the growing body of evidence that AVP is acting centrally as a neurotransmitter or neuromodulator to regulate body temperature during the febrile process.

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Dedicated to: Jack, Colleen, Louise and to Eli.

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I. INTRODUCTION

A. Thermoregulation

Maintenance of a constant internal environment is of vital importance to living organisms and is a fundamental axiom in biology. One of the variables of the internal environment that is of interest is that of temperature. Because of the temperature dependency of enzyme catalyzed chemical reactions, organisms strive to regulate the temperature at which these enzymatic and other chemical reactions occur. Regulation of the internal environment within an optimal temperature range therefore, increases an organism's fitness.

There are two general strategies which have evolved for the regulation of internal body temperature. Poikilotherms are those organisms that thermoregulate behaviorally by choosing a niche with a comfortable micro-environment. The amount of internal heat generated by the basal metabolic activity of poikilotherms coupled with the inability to prevent heat loss, prevents the maintenance of internal body temperature above the ambient temperature. Indeed, the basal metabolic rate for lizards is almost ten fold lower then placental mammals (Dawson, 1972). The body temperature of poikilotherms, therefore, conforms to the ambient conditions, whether that be an aqueous or terrestrial environment. Homeotherms, on the other hand, have evolved the ability to regulate their body temperature physiologically as well as behaviorally and, therefore, have a much wider range of habitats. Whatever the thermoregulatory strategy employed, the end result is the precise control of internal temperature within narrow limits peculiar to that organism.

Homeotherms have two basic processes for physiological regulation of body temperature; these are heat production and heat exchange (heat loss) mechanisms. The primary heat production effector available to homeothermic animals is the heat generated from basal metabolism. Since the energy exchange during chemical reactions produces heat energy, the sum total of the heat generated by these reactions in a rested, undisturbed and post-prandial subject is termed the basal metabolic rate (BMR) (Steinberg, 1985). Under these conditions the BMR can be equated to the heat production of the individual. However a number of factors can alter metabolic heat production including feeding, body size, age, ambient temperature and hormonal influences, among others. The temperature range in which metabolic rate is minimum and is independent of ambient temperature is termed the thermoneutral zone. The thermoneutral environment varies with the species. For an unclothed man thermoneutrality is approximately $30^{o}C$ (Stanier et al, 1984) whereas for the rat thermoneutrality it is at $26^{o}C$ (Stitt et al, 1985). Ambient temperatures below the thermoneutral zone result in conditions where the basal metabolism alone cannot maintain a stable deep body temperature. Under these circumstances, shivering, non-shivering thermogenesis (NST), heat loss reduction mechanisms as well as behavioral responses, provide additional ways to keep body temperature stable.

Shivering is the small rhythmic oscillatory contractions of muscles which produces heat by an increase in muscle tone, particularly of the jaws and limbs (Stanier et al, 1984). Thermosensory input from both the spinal cord and hypothalamus probably contribute in the control of shivering, at least in the rat (Banet et al, 1978) and guinea pig (Mercer and Simon, 1983).

NST is a process whereby heat is generated by the uncoupling of oxidation from phosphorylation reactions, particularly in cells of brown adipose tissue. NST is prevalent

in newborns (including humans) and cold acclimated animals. Brown fat is generally found in the subscapular regions and surrounding deep blood vessels of the neck. In the rat, initiation of NST is sympathetically controlled and has a lower threshold for activity compared to shivering (Banet and Hensel, 1974).

There are four modes of heat exchange between an organism and the environment: radiation, conduction, convection and evaporation. Radiation is the process where an organism may lose or gain heat from the environment depending on the direction of the thermal gradient. Radiation is a passive process which can either be working to increase or decrease core temperature. Radiation is not under physiological control.

Conduction is the transfer of heat between two bodies in contact. The direction of heat transfer is dependent on the direction of the thermal gradient between the two bodies. Conduction can be controlled behaviorally by minimizing or maximizing the amount of surface area in contact.

Convection is the movement of heat, via the air or water, from a body surface to the environment. This includes the transference of heat from the body core to the periphery via the circulation. Convection can be controlled primarily through alterations in cutaneous vasomotor tone and by behavioral means.Radiation, conduction and convection are all non-evaporative means of heat loss; although radiation and conduction can also be heat gain processes.

Evaporation describes the loss of heat due to a humidity gradient when the temperature gradient is small or reversed. The evaporation of sweat from the surface of the skin results in latent heat being taken up at 2400 J/g, with consequent cooling of the body (Stanier et al, 1984). This is important during conditions where ambient temperature

approaches body temperature, since convective heat loss under these circumstances is ineffectual. Panting and sweating are the two principal modes of evaporative heat loss. A behavioral adaptation to increase evaporative heat loss is often observed in fur bearing animals whereby the spreading of saliva onto the fur enhances evaporative heat dissipation.

The reduction of heat loss during cold exposure is accomplished by cutaneous vasoconstriction. The reduction in blood flow to the skin reduces heat loss by non-evaporative means. It is generally accepted that peripheral vasoconstriction in response to body cooling is generated by posterior hypothalamic initiation of sympathetic activity (Guyton, 1980). Other adaptations such as increases in thermal insulation via fur or feathers and subcutaneous fat contribute to the lessening of heat loss. In fur bearing animals piloerection conserves heat by forming a layer of insulating air between the environment and skin. Finally, behavioral adjustments such as a huddled posture will minimize exposed surface area and thereby reduce heat loss.

The response to a decrease in ambient temperature is a variety of adaptive responses to counter the tendency for a reduced body temperature. A similar situation exists in the opposite direction, namely, there are appropriate responses to maintain body temperature in the face of excess heat. During heat stress, convection and evaporation are controlled physiologically and are therefore of greatest importance.

From the above discussion it is evident that there are many ways available for homeotherms to make adjustments in heat production or heat loss. This has obvious adaptive advantages in the face of thermal stimuli which would otherwise cause a change in core temperature. To make the necessary adjustments sensory information must be relayed from the thermosensors to the brain. These thermosensors are found in the skin,

deep body viscera, spinal cord, brain stem and hypothalamus. The common property of these thermosensors is that they are neural elements which transduce thermal stimuli into coded neural impulses ultimately inducing a thermoregulatory response.

Two classes of cutaneous thermosensors have been found in fur bearing animals (Hammel, 1972) as well as humans (Simon et al, 1986). Peripheral warm and cold "receptors" are undefined structures in free nerve endings of A-delta or C fibres (Martin, 1983). Cold and warm responsive fibres have both phasic and tonic discharge characteristics. That is, they have a static discharge at constant temperatures and show activity bursts during temperature changes (Hensel, 1973). The static activity characteristics of both cold and warm "receptors" as a function of temperature forms a bell shaped curve where the maximum firing rates of each receptor is temperature- and species-specific (Hammel, 1972). Although the mechanisms of temperature transduction are not fully understood, experimental evidence indicates the importance of the Na^+-K^+-ATPase (Pierau, 1975) and external $[Ca^{2+}]$ (Hensel and Schafer, 1974) on receptor potential generation in cutaneous temperature sensors. Analysis of thermoafferent pathways in cutaneous thermosensation has concentrated mainly on the trigeminal (face) and inguinal (scrotum) regions. Input to trigeminal neurons is relayed, without significant processing, in the trigeminal nucleus caudalis. Here the neurons form small receptive fields which are strictly ipsilateral (Simon et al, 1986).

Thermal afferents of the spinal cord have been well studied. Afferent information is relayed via the dorsal horn, where information processing can occur through local or descending inputs, and ascends in the spinothalamic tract. In addition, the receptive fields of these spinal structures are large and bilaterally represented, indicating extensive convergence of afferent information (Simon et al, 1986).

Thermosensors in the spinal cord were first suggested in studies where localized spinal cord cooling of animals with intact as well as bilaterally sectioned dorsal roots resulted in shivering (Thauer, 1970). Local heating or cooling of the spinal cord can evoke appropriate peripheral vasomotor responses. Cooling the spinal cord, for example, produces vasoconstriction while warming the cord causes vasodilation (Chai and Lin, 1972). Studies have also shown that thermosensitive structures within the spinal cord are involved in heat loss responses such as panting (Boulant, 1980). Thermosensitive spinal cord neurons have been investigated electrophysiologically and have similar characteristics as cutaneous warm and cold receptors, suggesting that spinal cord neurons have primary thermoreceptor capability (Klussman and Pierau, 1972).

A continuation of ascending thermosensitivity is manifest in the role of the brain stem in deep body thermosensation. Lesion and thermode studies have shown that neural structures within the pons and medulla are involved in appropriate heat production (shivering and NST) and heat loss (panting) responses (Boulant, 1980). In addition, single unit studies incorporating midbrain raphe nuclei and medullary neurons indicate that these neurons are thermoresponsive to peripheral (Jahns, 1976) as well as local temperature changes (Watanabe et al, 1986). These brainstem structures have divergent fibre projections to the reticular formation (Jahns, 1976), sensory thalamic nuclei as well as to the regulatory hypothalamic areas (Hellon and Taylor, 1982).

Non-CNS deep body thermosensors also appear to contribute to a multiple-input concept for thermosensation. Appropriate thermoregulatory responses have been observed during thermal stimulation of abdominal viscera (Rawson and Quick, 1970), vagal and splanchnic afferents as well as structures of the skeletomuscular system (Boulant and Dean, 1986).

Although the role of non-CNS deep body thermosensors is unclear, there appears to be a CNS hierarchy of thermoregulatory control where the hypothalamus, particularly the preoptic anterior hypothalamus (POAH), has a dominant role. A vast amount of research has been devoted to the investigation of the POAH in thermoregulation. Lesion and thermode studies clearly indicate that the POAH is involved in the control and integration of thermoregulatory processes. Single unit investigation of this area began in 1961, when Nakayama first identified neurons in the hypothalamus which were temperature sensitive beyond that expected from the Arrhenius equation. Electrophysiological study of the POAH both in vivo and in vitro continues to this day.

For neurons to be considered temperature-sensitive the slope of the firing rate-temperature response curve must meet certain criteria (Boulant, 1980; Nakayama, 1985). Based on these criteria, three groups of neurons are present in the POAH: warm, cold and temperature-insensitive. Analysis of these temperature-sensitive neurons in slice preparations (Hori et al, 1980; Kelso et al, 1982) and in culture (Baldino and Geller, 1982; Hori and Nakayama, 1982) indicates that these neurons have an inherent thermosensitivity. That is, afferent input does not determine neuronal thermosensitivity.

The lack of extensive intracellular investigation of POAH neurons (Nelson and Prosser, 1981) precludes speculation on the membrane characteristics contributing to the thermosensitivity of these neurons. Limited morphological studies have shown, at least in the fish, that POAH warm-sensitive neurons receive varying degrees of synaptic input while cold-sensitive neurons consistently receive much more synaptic input (Boulant, 1986). This information provides support for the hypothesis that cold-sensitive neurons derive this characteristic from synaptic input of warm-sensitive neurons (Boulant, 1981).

Integration of central and peripheral thermal information undoubtedly occurs in the POAH. Studies have shown, using in vivo single unit recordings, that POAH temperature-sensitive neurons receive varying degrees of afferent input and that the firing rates of these neurons is a function of the amount of afferent input from the skin, spinal cord and brain stem (Guieu and Hardy, 1970; Hellon, 1972; Boulant and Hardy, 1974; Boulant, 1981). In addition, the firing rates of warm- and cold-sensitive neurons during changes in POAH temperature produce curves which are similar to heat loss and heat production responses observed during similar POAH temperature manipulations (Boulant, 1986). Integration of thermal information is thought to be due to competitive summation of local and peripheral inputs to warm-sensitive neurons which in turn synaptically influence cold-sensitive neurons (Boulant, 1981). Although animals with POAH lesions have the ability to thermoregulate fairly normally no other area (brain stem or spinal cord) has as great a thermosensitivity as the POAH. Based on this apparent hierarchy and the evidence of central integration within the POAH, this neural area is considered as the primary centre for thermoregulation (Boulant, 1980; Boulant, 1981; Hensel, 1981).

Although the POAH appears to have a prominent role in the thermoregulatory process, the generation of a reference signal or set-point around which core temperature is regulated remains to be elucidated in terms of structure and physiological processes. Several neuronal models have been proposed, based on single unit data, to account for the set-point determination. Hammel (1965) was the first to propose a hypothesis for hypothalamic control of set-point temperature. This model incorporated both temperature-sensitive and temperature-insensitive neurons as well as effector set-point interneurons. These interneurons were the apparent determinant for the set-point. However, 20 years of investigation has led to modifications of Hammel's proposal (Bligh, 1972; Boulant, 1980), particularly in lieu of the lack of evidence supporting the existence of set-point interneurons. The predominant view is that a physiological correlate for a reference signal

does not exist and that the set-point can be established by two opposing inputs, viz: warm- and cold-sensitive neurons (Mitchell et al, 1970; Bligh, 1972). Thus the point of intersection between the firing rates of the two neuronal populations could explain the existence of the body temperature set-point without a reference signal. This is supported by single unit studies employing various pyrogens, where the sensitivity or gain of the neuronal responses to temperature changes after exposure to pyrogenic agents (Wit and Wang, 1968; Eisenman, 1969; Boulant and Scott, 1983; Hori et al, 1984; Nakashima et al, 1985). This change in sensitivity of POAH neurons can adequately account for the set-point determination and the so called set-point shift during fever.

An alternative hypothesis for the determination of the body temperature set-point was proposed by Myers and Veale (1970; 1971). These authors implicated the posterior hypothalamus as a relay point for efferent signals arising from the POAH. Based on their evidence they proposed that the ratio of Na^+ to Ca^{2+} in the area of the posterior hypothalamus was responsible for determining the temperature set-point by influencing neurons in that area. Measurements of the $Na^+:Ca^{2+}$ in the cerebral extracellular fluid in normal and febrile states remains to be done to corroborate the hypothesis.

B. Fever

A pathological alteration of the thermoregulatory system in response to infection or inflammation is fever. Fever has been recognized as a consequence of inflammation since the 6th century B.C. and as a factor in infection since 450 B.C. (Veale et al, 1984). In ancient Greece, Hippocrates recognized fever as a sign of infection and described the symptoms in such detail that a diagnosis of malaria could be made from his treatises (Dinarello and Wolff, 1980). In addition to his observations, Hippocrates postulated a mechanism for the production of fever based on the four humors: blood, phlegm, black bile

and yellow bile. To the ancient Greeks, fever was due to an excess of yellow bile since its qualities were hot and dry (Dinarello and Wolff, 1980). The humoral view of fever, and disease in general, was the cornerstone of medical thinking for nearly 2000 years. In the late 19th century Liebermiester postulated that fever was due to a resetting of the body temperature to a new higher level (Atkins and Bodel, 1979). Shortly after, William Welch presented a classic paper which espoused the modern concept of fever. Welch stated that the central nervous system controlled body temperature in febrile as well as non-febrile states. He defined this central structure as sub-cortical and suggested that fever was generated by the action of "leukocyte ferment" on this structure (Atkins and Bodel, 1979).

Fever is recognized as a pathological process whereby the core temperature is elevated usually as a result of some pyrogenic agent. Fever is distinguished from hyperthermia on the basis that the elevated temperatures observed during fever are due to a functional thermoregulatory response which maintains the elevated temperature. Hyperthermia, on the other hand, is an uncompensated disequilibrium of heat balance (Stitt, 1979). Because the temperature elevation during a febrile episode is defended by appropriate thermoregulatory responses, fever is considered to be due to an elevated body temperature set-point (Cooper et al, 1964; Cooper, 1972; Cabanac and Massonet, 1974). Single unit studies support this contention (Wit and Wang, 1968; Eisenman, 1969; Boulant and Scott, 1983; Hori et al, 1984; Nakashima et al, 1985), as do studies involving reptiles and amphibians (Kluger, 1979). If fever is considered as an elevation in the temperature set-point, then it is possible for both hypothermia (a core temperature below the set-point) or hyperthermia (a core temperature above the set-point) to occur in febrile organisms. Indeed, an explanation for the so called "chill phase" at the onset of fever is that the body temperature set-point, having been elevated, puts the subject in a hypothermic state and therefore the preferred ambient temperature is higher. Thus the individual feels subjectively cold (Stitt, 1979).

As mentioned previously, fever can be initiated by infectious agents; examples of which are: gram negative and positive bacteria, viruses, fungi and protozoa. Additionally fever is also a symptom in certain malignancies, non-specific and immunological conditions as well as non-infective inflammation (Hellon and Townsend, 1983). Bacterial pyrogens, specifically gram negative bacteria, derive their pyrogenicity from the composition of their cell walls.

Endotoxins, derived from the cell walls of gram negative bacteria, are ubiquitous in nature because they are constantly shed into the environment by the bacterium. In an unpurified form endotoxin contains lipid, carbohydrate and protein. However, purified endotoxin, such as used for research purposes, contains lipopolysaccharide of molecular weight around 2 million (Pearson, 1985). There are three primary components to the endotoxin molecule: (i) a core region of polysaccharides (ii) an O-specific side chain, and (iii) a lipid moiety which gives the endotoxin its pyrogenicity (Veale et al, 1984). Endotoxin is used extensively in fever research primarily because the febrile responses are not lethal, they are repeatable and the endotoxin is activated in a similar manner as with live bacteria (Kasting et al, 1982).

Exogenous pyrogens, such as endotoxin, produce fever via a cascade of events initially involving cells of the reticuloendothelial system (RES). The cells of the RES are immunologically active phagocytes found throughout the body which produce a heat labile protein called endogenous pyrogen (EP). Extensive research on EP has shown that this molecule is the same or very closely related to other mediators of the acute-phase response to infection. Indeed the term interleukin-1 (IL-1) is used to describe not only EP but a family of closely related molecules perhaps sharing a common gene sequence and expressing multiple biologic activities important for host defense (Dinarello, 1984). EP (or

IL-1) has been shown to be produced in mammals, reptiles and amphibians (Kluger, 1979) and exhibits considerable pyrogenic cross-reactivity amongst species (Hellon and Townsend, 1983).

Circulating EP reaches the brain via the cerebral vasculature where its site of action is thought to be at (Eisenman, 1969; Dinarello, 1984; Schoner and Wang, 1975; Wit and Wang, 1968) or near (Stitt ,1986) the POAH. The lack of evidence that EP can actually cross the blood brain barrier suggests that it is unlikely that the pyrogen (a 15000 Dalton polypeptide) acts directly on the POAH to initiate fever. Although EP produces fever when administered intravenously, intracerebrally (i.e. POAH) and intracerebroventricularly (icv) it is thought that circulating EP acts on target cells outside the blood brain barrier probably in the circumventricular structure called the organum vasculosum laminae terminalis (OVLT) (Blatteis et al, 1983; Stitt, 1985; Stitt, 1986). The vasculature in the area of the OVLT lacks the typical endothelial tight junctions characteristic of the blood brain barrier. Therefore the passage of large molecular weight proteins, such as EP, is possible within this neural area. The target cell mediating the effects of circulating EP has been suggested to be a mesenchymally derived phagocyte located in the perivascular space of the OVLT (Stitt, 1986). The action of EP when administered within the POAH or icv is thought to be a non-specific effect of EP since the pyrogen can neither be detected within the POAH or cerebrospinal fluid during febrile episodes (Stitt, 1986).

Since it seems unlikely that circulating EP crosses the blood brain barrier, the existence of a mediator which translates the appropriate signals from the OVLT, across the blood brain barrier and to the POAH is necessary. The central mediators of fever that have generated much study and controversy are the prostaglandins of the E series.

Circulating EP probably causes fever by first inducing the synthesis of PGE which in turn stimulates the temperature-sensitive neurons of the POAH (Stitt, 1986; Bernheim, 1986; Coceani et al, 1986).

Microinjection of PGE_2 into the ventricular system of the cat, rat or rabbit produces dose dependent increases in rectal temperature which have very similar dose-response sensitivities. These observations suggest a common fever-producing pathway is activated in all three species of animals (Stitt, 1986). Experiments sampling the CSF of febrile (endotoxin- or EP-treated) animals have demonstrated PGE_2 release into the CSF is enhanced compared to non-febrile animals (Feldberg and Gupta, 1973; Bernheim et al, 1980; Coceani et al, 1983). In addition, prostaglandin synthesis inhibitors are all effective antipyretic agents and can reverse febrile levels of CSF PGE_2 (Feldberg et al, 1973; Dey et al, 1974a). While many neuronal cell types have been shown to produce PGE in response to EP in vitro, the target cells for circulating EP are considered as the likely candidates for PGE production during normal fever development (Stitt, 1986). It should be noted that the prostaglandin-fever issue is not without controversy and evidence unsupportive of the views stated above has been published (Mitchell et al, 1986).

Several neurotransmitters or other mediating substances have been reported to be involved in the central pathways subserving fever regulation. In addition to the prostaglandins, monoamines, acetylcholine, cyclic nucleotides and Na^+ and Ca^{2+} all may play a role mediating the effects of circulating EP.

The question of whether fever has adaptive value is a controversial issue. The ability to respond to infection with fever is a characteristic which is phylogenetically conserved. Invertebrates, fish, reptiles and amphibians all respond to infection by moving to an area in which the ambient temperature raises the body temperature to febrile levels

(Kasting et al, 1982; Kluger, 1979). Indeed, experiments by Kluger and his co-workers have shown that in reptiles and amphibians the ability to raise body temperature during infection is critical for survival (Kluger, 1979).

Birds and mammals also respond to infection with elevated body temperatures. Whether fever is important for survival in mammals is unclear. A study in rabbits by Kluger and Vaughn (1978) found that fever height up to $2.25^{\circ}C$ was correlated with survival. However, febrile temperatures above $2.25^{\circ}C$ were associated with a decrease in survival. Banet (1979) presented evidence from studies in the rat which supports Kluger and Vaughn in that excessively high fevers tends to cause a decline in survival. The widespread use of antipyretic drugs in humans suggests that elevated body temperatures are not necessary for enhanced survival or recovery from infection (Kasting et al, 1982). Indeed the risk of febrile convulsions in children would indicate the seriousness of high fevers and provides a rationale for antipyretic intervention.

C. Antipyretic Drugs

Antipyretics are drugs which decrease febrile temperatures but have no effect on non-febrile body temperatures unless the dose is excessive (Barbour, 1921). Antipyretics can only alleviate the symptom (fever) but can not eliminate the cause (infection). Thus antibiotics are not considered as antipyretic drugs (Kasting et al, 1982). Figure 1 illustrates the chemical structures of three common antipyretic drugs.

Antipyretics could act in a number of ways to counter febrile increases in temperature (Van Arman et al, 1985):

(1) antipyretics could prevent EP production

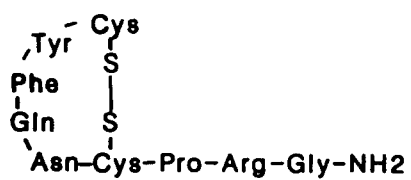(2) antipyretics could block EP at its neural target cell

FIGURE 1

Chemical structures of three common non-steroidal antipyretic drugs. A. Acetylsalicylic acid B. Acetaminophen C. Indomethacin

A

B

Tyr — Cys
Phe   S
Gln   S
Asn—Cys-Pro-Arg-Gly-NH2
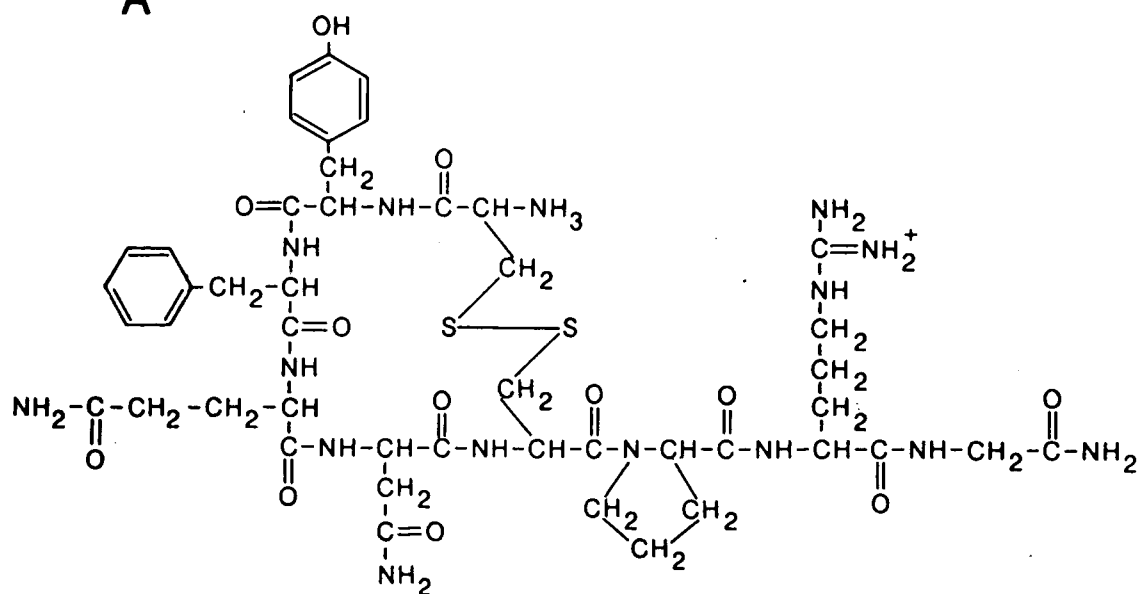
(3) antipyretics could prevent release or synthesis of prostaglandins

(4) antipyretics could act on central neurons driving thermoregulatory actions

(5) antipyretics could act directly on effector organs

Several studies using in vitro as well as in vivo techniques have shown that antipyretics have no effect on EP production or release (Clark and Cumby, 1975; Clark and Moyer, 1972). Although antipyretic treatments can reverse EP-induced fever the validity of point (2) is questionable since EP probably does not have access to the neuropil. Point (4) is possible since antipyretics have been shown to reverse pyrogen-stimulated single units within the POAH (Wit and Wang, 1968; Eisenman, 1969; Schoener and Wang, 1975). The question of whether antipyretics act directly on effector mechanisms has been studied in cold exposed animals. The results indicate that antipyretics do not act directly on effectors (Cranston et al, 1975; Pittman et al, 1976). Although there is some evidence in both rats (Satinoff, 1972; Francesconi and Mager, 1975) and cats (Cranston et al, 1975) to the contrary.

The most likely mechanism by which antipyretics act is through inhibition of prostaglandin synthesis. Antipyretics, such as illustrated in Figure 1, are potent inhibitors of prostaglandin synthesis primarily through the inactivation of the cyclooxygenase enzyme (Vane, 1971). The ability of an antipyretic to reduce fever is proportional to its ability to inhibit prostaglandin production (Dinarello, 1984). This is supported by the data of Feldberg et al (1973) which demonstrated that acetaminophen, acetylsalicylic acid and indomethacin reversed febrile levels of CSF PGE_2. In support of this Dey et al (1974b) found that PGE_2 could also be reduced by administration of the same antipyretic drugs to the febrile cat. It is unknown, however, whether prostaglandins play a role in fever genesis in man, despite the effectiveness of antipyretic therapies.
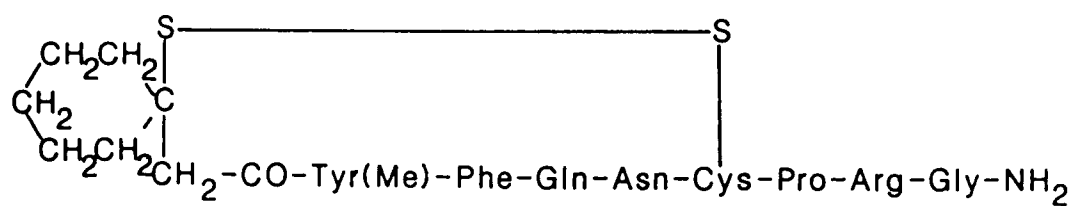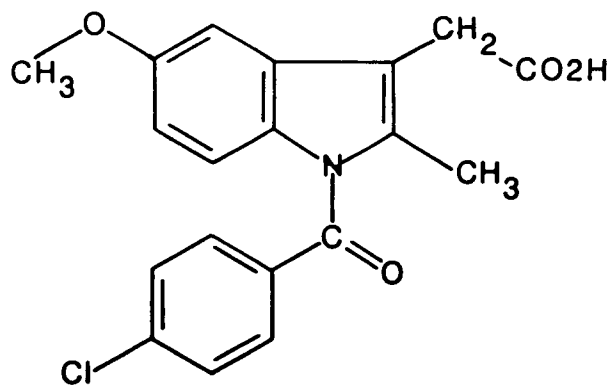
D. Endogenous Antipyresis

Antipyretic drugs are useful in reducing febrile temperatures where the danger of excessively high fevers is possible. Studies in the rabbit (Kluger and Vaughn, 1978) and the rat (Banet, 1979) demonstrate the potential lethal effects of high febrile temperatures. In addition, the possibility of febrile convulsions in children all provide evidence warranting the use of antipyretic drugs. Since the late 70's the concept of an endogenously released antipyretic substance has been hypothesized based on investigations in periparturient animals and their newborn as well as in non-pregnant animals. This concept is further supported by neuroanatomical data, receptor studies and a variety of in vivo techniques all which point to the neuropeptide arginine vasopressin (AVP) as a putative neurotransmitter substance involved in fever regulation and/or suppression.

AVP is a nonapeptide (Figure 2A and 2B) which has been recognized as a hormone involved in both water balance and blood pressure regulation. Increases in plasma osmolality or hypovolemia stimulate AVP release from the neurohypophysis into the plasma. The hormone then acts on the kidney tubules to increase water reabsorption via V_2-receptors (Butlen et al, 1978). The cardiovascular effects of AVP are mediated through the V_1-receptor located in the vascular smooth muscle (Michell et al, 1979). The source of peripherally released AVP is primarily the supraoptic nucleus (SON) located in the hypothalamus (Sofroniew and Wiendl, 1981). However, other vasopressin-containing nuclei with extensive projections throughout the brain suggest that AVP may be a neuropeptide transmitter substance.

Work by Buijs (1978) and associates (Buijs et al, 1978; Buijs and Swaab, 1979) have demonstrated extensive intra- and extrahypothalamic vasopressin pathways in the rat brain. In addition to being present in the paraventricular nucleus (PVN), suprachiasmatic nucleus (SCN) and SON, AVP is found in pathways from the PVN to the

FIGURE 2

Arginine vasopressin (AVP) and the AVP antagonist (M-AVP). A. Chemical structure of AVP B. Amino acid sequence of AVP C. Amino acid sequence of M-AVP.

A

$$S\text{---------------------------}S$$

CH$_2$-CO-Tyr(Me)-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH$_2$

B

CH$_2$-CO2H

O-CH$_3$

CH$_3$

N

C=O

Cl

lateral and medial septum, substantia gelatinosa, substantia nigra, hippocampus and the nucleus tractus solitarius. Pathways from the SCN project to the OVLT, PVN, SON, septum and diagonal band area (Sofroniew and Wiendl, 1981). Furthermore, extrahypothalamic AVP-containing nuclei, for example the bed nucleus of the stria terminalis (BST), locus ceruleus and amygdala (Van Leeuwen and Caffe, 1983; DeVries et al, 1985) are important sources for centrally released vasopressin.

Further investigation has shown that AVP is contained within synaptic vesicles (Buijs and Swaab, 1979) and is released into the CSF in concentrations not related to those in the blood (Dogterom et al, 1977; Mens et al, 1982). Furthermore vasopressin released into the neuropil has been measured in vivo in conscious (Cooper et al, 1979; Ruwe et al, 1985a) and anaesthetized animals (Demotes-Mainard et al, 1986) using the technique of push-pull perfusion. Vasopressin release has also been demonstrated in vitro where the process is K^+-stimulated and Ca^{2+}-dependent (Buijs and Van Heerikhuize, 1982). The above evidence, together with receptor studies (Baskin et al, 1983; Biegon et al, 1984; DeWeid et al, 1984), all point to the likelihood that centrally released AVP is acting as a neurotransmitter/neuromodulator within the brain.

Studies which investigated the development of fever in the newborn eventually led to the hypothesis that AVP was involved in endogenous antipyresis. It had been observed for some time that newborn humans did not respond to bacterial infection with fever (Kasting et al, 1980a). To investigate this phenomenon newborn lambs were chosen as a model to study the ontogeny of fever mainly because of their thermoregulatory maturity at birth. Pittman et al (1974) demonstrated that lambs receiving an iv injection of bacterial endotoxin 4 hours or 60 hours after birth failed to develop fever. But lambs receiving endotoxin 4 hours and then 60 hours post partum did become febrile. It was evident from this that there was a sensitizing effect of a previous exposure to endotoxin in the newborn.

Subsequent studies showed that the sensitizing process could not be initiated by endotoxin injections in utero (Pittman et al, 1974) and that the near and postpartum ewe were unresponsive to endotoxin challenges (Kasting et al, 1978). A possible explanation for these observations was that the cells of the RES were not responding to endotoxin with the production of EP. This hypothesis was tested by Kasting et al (1979b) who showed that, in vitro, the white blood cells of both the fetus and the ewe were capable of responding to endotoxin with the production of EP. The EP produced in these experiments demonstrated all the characteristics of EP from non-pregnant controls. It was suggested from this that there may be an active inhibition of fever at the level of the central nervous system.

A search for a circulating substance which might be involved in fever inhibition was subsequently initiated using three criteria (Kasting et al, 1980a). The first was that the circulating concentration of the substance should increase in both the ewe and the fetus. Secondly, the increase should start about four days before term and peak at term. The last criterion was that the substance should return to normal by about 32 hours postpartum. The substance which best fit these criteria was AVP.

Further investigations into near term fever suppression was carried out in guinea pigs by Zeisberger et al (1981). These investigators demonstrated that the periparturient guinea pig also shows a diminished febrile response to endotoxin challenge. This suppression was evident at parturition in mother animals as well as newborns. Immunocytochemical studies on the pregnant guinea pigs demonstrated an increase in AVP-containing neuronal elements in the septohypothalamic system and amygdalohypothalamic system (Merker et al, 1981). These changes lasted until one day postpartum when normal AVP staining was observed. In the new born rat pup the ability to respond to endotoxin with fever is also suppressed. However, if a specific AVP V_1-antagonist (see Figure 2C) is administered to these animals they are able to respond to

endotoxin by generating a fever behaviorally (Kasting and Wilkinson, 1987). There is therefore, strong evidence linking the neuropeptide AVP in the suppression of fever in near term animals and their newborn.

The antipyretic action of AVP is not restricted to pregnant and new born animals. Kasting et al (1979c) investigated the antipyretic effects of AVP in non-pregnant sheep using push-pull perfusion. After considerable exploration of the brain using this technique it was found that AVP perfusions into the ventral septal area (VSA) of the sheep could suppress fever induced by endotoxin. Furthermore perfusion of the VSA in non-febrile animals had no effect on body temperature. In a subsequent study (Cooper et al, 1979) a negative correlation was found between fever height and plasma AVP levels. That is, when AVP release was high, little or no fever developed and when AVP was low, normal or near normal fevers resulted. A similar correlation was found in the push-pull perfusates of the VSA. Thus not only was the VSA sensitive to exogenously administered AVP, but that during fever AVP was being released into the extracellular fluid of this neural area.

Continued study of the VSA, using push-pull perfusion (Kasting, 1980), demonstrated that this area was sensitive only to AVP. Other peptides such as oxytocin, somatostatin, angiotensin II and substance P all had no effect on body temperature when perfused within the VSA. Furthermore, perfusion of either an AVP-antisera or an AVP analogue (desaminodicarba-AVP) into the VSA enhanced the febrile response to iv endotoxin while having no effect in the non-febrile animal. The VSA is richly endowed with AVP-terminals (Sofroniew and Wiendl, 1981; Van Leeuwen and Caffe, 1983; DeVries et al, 1985) and AVP receptors (Baskin et al, 1983). This provides strong evidence that the VSA is the neural area where vasopressin is mediating its antipyretic effects.

Investigations on the antipyretic effects of AVP have also been carried out in the rabbit (Naylor et al, 1985), rat (Ruwe et al, 1985) and cat (Naylor et al, 1986). In each instance AVP perfusions within the VSA suppressed the onset of endotoxin- or prostaglandin-induced fever. Furthermore, Cooper et al (1987) have shown that EP-induced fever in the rat can be enhanced by the administration of a specific AVP V_1- but not V_2-antagonist within the VSA. Since this same V_1-antagonist can prevent the antipyretic effect of AVP icv (Kasting and Wilkinson, 1986) it is likely that endogenous or exogenous AVP is acting at a V_1-like receptor in the brain to modulate fever.

Methods employed to stimulate endogenous vasopressin release can also mediate antipyresis. Hemorrhage of 20% of the blood volume in sheep suppressed the onset of fever following iv endotoxin (Kasting et al, 1981), while having no effect in non-febrile controls. In the rat, hemorrhage also mediates antipyresis in an established endotoxin fever (Kasting, 1986a). In addition, this study showed that hypertonic saline, delivered iv, was also an antipyretic stimulus. It was concluded from these data that centrally released AVP was mediating the observed antipyresis. This conclusion is supported from studies where hemorrhage and hypertonic saline both increased AVP release into the CSF (Barnard and Morris, 1982; Szczepanska-Sadowska et al, 1983; Szczepanska-Sadowska et al, 1984; Wang et al, 1985). Moreover, elevated plasma osmolality results in increased AVP release within the neuropil (Ruwe et al, 1985; Demotes-Mainard et al, 1986).

E. A Rationale for Investigation

Series 1. AVP-Antagonist

A study by Kasting et al (1980b) demonstrated that in the non-febrile rat icv administration of vasopressin had hypothermic and convulsive effects. The results of this study provided the initial impetus for developing a rat model to study the antipyretic effects of AVP administered into the ventricular system during an established endotoxin fever. In addition, the icv administration of the V_1-antagonist would be employed to determine the specificity of the AVP-induced response.

Series 2. AVP at Different Ambient Temperatures

It was hoped, having established the potent antipyretic effects of icv AVP in Series 1, that the effector mechanisms involved in the AVP-induced antipyresis could be elucidated. This would be attempted by exposing febrile and non-febrile rats to cold ($4^{\circ}C$), neutral ($25^{\circ}C$) and warm ($32^{\circ}C$) ambient temperatures. Acute warm exposure will stimulate heat loss effectors and minimize heat production while acute cold exposure will stimulate heat production and minimize heat loss effectors. Indomethacin, a widely used and effective antipyretic drug (Clark and Cumby, 1975) was used for a comparative evaluation of the antipyretic effects of AVP.

Series 3. Thermoregulatory Effector Mechanisms

Measurements of specific heat loss and heat production effectors were carried out to further evaluate the mechanism of AVP-induced antipyresis at different ambient temperatures.

Series 4. AVP Levels in the CSF

Determination of CSF levels of AVP were conducted in febrile and non-febrile rats in order to assess whether endogenously released AVP might be detectable in CSF during fever or use CSF as a conduit for transportation to its site of action.

II. METHODS

A. Cannula Construction

All guide and injection cannulae were prepared from stainless steel hypodermic tubing (Small Parts Inc.). Intracerebroventricular (icv) guide cannulae were constructed from 23 gauge tubing cut to a length of 15 mm and bevelled at one end with a grindstone. Halfway up the cannula a small cross bar was soldered to facilitate anchoring the cannula in the subsequently applied dental acrylic. A 30 gauge stylet was used to keep the guide tube clean and patent. Injection cannulae were also made from 30 gauge tubing which had a 23 gauge sleeve soldered on near one end thereby allowing a pre-determined length (17.5 mm) to be lowered into the guide tube. Injection cannulae were connected by a length of PE-20 tubing, filled with the appropriate injectate, to a Hamilton microsyringe (10 uL).

For CSF withdrawal, cannulae were cut from 21 gauge hypodermic needles. The tip which was to be inserted, was ground to a 45 degree angle with a grindstone. The other end was ground smooth. The total cannula length was 23 mm. A small cross piece was soldered 8 mm from the tip so that the cannula shaft could be inserted only up to this point. Stylets were manufactured from 26 gauge needles which had a 21 gauge sleeve at the outer tip and were bevelled flush with the 21 gauge tube. A lump of solder occluded and sealed the top end of the stylet to prevent CSF leakage and possible contamination. A PE-100 sleeve was used on both the stylet cap and a portion of the exposed end of the cannula, to seal the cannula-stylet assembly.

B. Experimental Series 1. AVP Antagonist

Male Wistar rats (250-350 g) were used in all experiments. Under pentobarbital anaesthesia (50 mg/kg, i.p.) rats were stereotaxically implanted with a 23 gauge guide cannula directed towards the left lateral ventricle according to the coordinates of Pellegrino et al (1981), (0.4 mm posterior to bregma; 2.0 mm lateral to the midline and 2.5 mm below the skull). The guide cannula was fitted with a 30 gauge stylet of corresponding length. Placement of the icv cannula was verified by injection of 5 uL of bromophenol blue dye (Sigma) and gross histologic examination of stained ventricles. An individually calibrated glass thermistor (Fenwal) was implanted caudally to the guide tube and lowered 3 mm from dura. This placed the thermistor tip in the region of the caudate-putamen. Prior to the cannula placement, 5 evenly spaced holes were drilled into the exposed skull to accommodate stainless steel jewellers screws. The guide cannula and thermistor were then implanted and secured by the application of dental acrylic which flowed around the screws, cannula and thermistor. To protect these devices, a paper clip bent to 90 degrees and equipped with a snap fastener soldered to it, was positioned and also cemented to the skull of the rat. The corresponding end of the snap fastener was soldered to the end of a steel spring and ball bearing assembly at the top of the cage. This arrangement allowed for unrestricted movement of the rat as well as providing a protective avenue for thermistor wires or PE tubing leading to the outside of the cage (Figure 3). Indomethacin-treated animals were also implanted with a Silastic catheter passed subcutaneously along the back. This catheter was exteriorized via the skull using a 20 gauge stainless steel "U"-tube, the free end of which was connected, by PE-100 tubing, to a 1.0 mL syringe situated outside the cage.

The experimental cage consisted of standard wire mesh containing the spring and ball-bearing assembly (Figure 3). This wire cage was enclosed within an isolation box

FIGURE 3

Diagram illustrating the tethering system employed for each experiment. This method allowed the animal to move about freely while protecting thermistor wires and/or PE tubing.

Roller Bearing Assembly
(Cross Section)

Steel Spring

Paper Clip

Snap Fastener

Thermistor

ICV Cannula
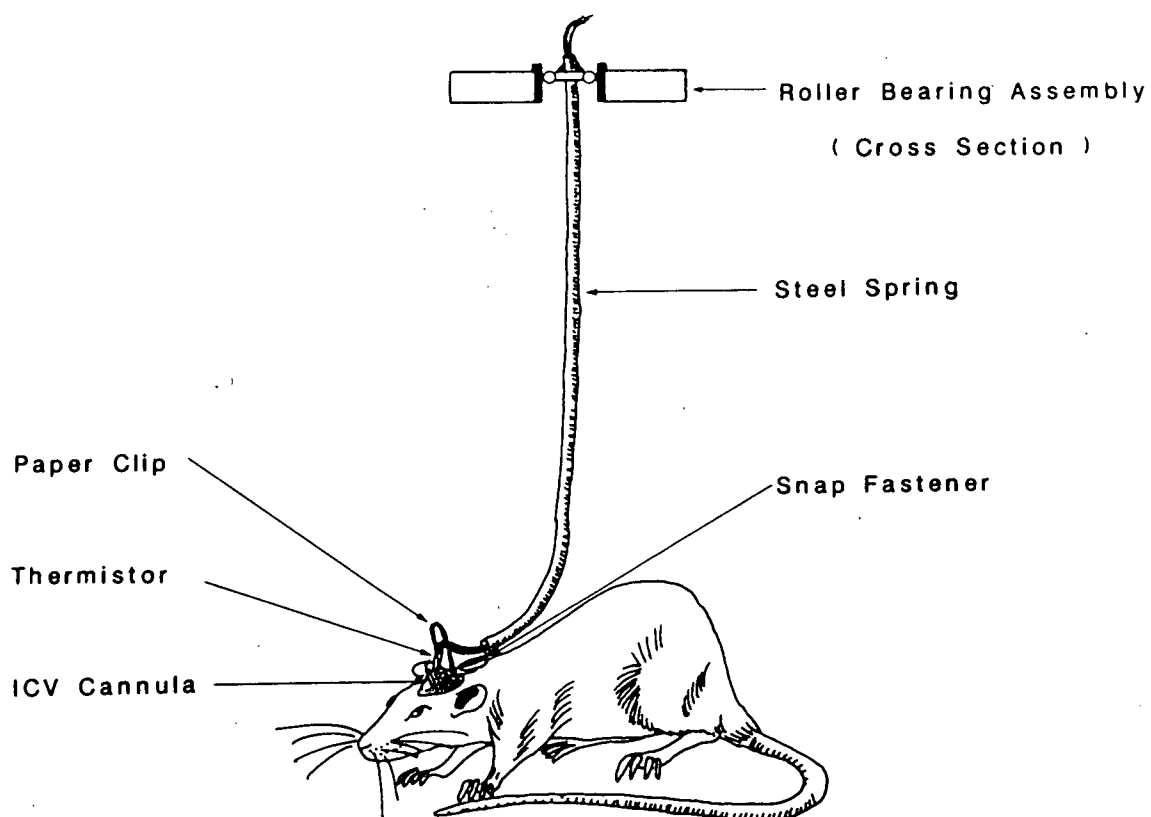
equipped with a 9 V fan and two 8 W fluorescent lights for air circulation and illumination respectively. Lighting was automatically controlled to a 12:12 light-dark schedule. Food and water were available ad libitum. All experiments were performed at an ambient temperature of $25 \pm 1^{\circ}$C.

Prior to starting an experiment brain temperatures were monitored for at least 60 min in order to establish that a stable baseline existed. Following this, 10 ng of endotoxin (Escherichia coli, lipopolysaccharide, Sigma) was microinjected icv. All icv injections were made up to 5 uL using sterile physiological saline delivered over a 2 min period. The resulting febrile rise in brain temperature was monitored for 150 min. At this point vehicle (0.9% sterile saline) or AVP (5.0 ng, Bachem) were injected icv and the resulting temperature changes followed for 60 min (see Figure 5).

The AVP V_1-receptor antagonist, [1-(B-mercapto-B,B-cyclopentamethylene propionic acid)-2-(O-methyl) tyrosine] arginine vasopressin (Kruszynski et al, 1980), abbreviated M-AVP, was administered icv (1 uL/ug) 2 min or 24 h before AVP in the febrile rat. As a control, saline (5 uL) was administered 2 min prior to AVP. In addition both AVP and M-AVP were each injected separately in non-febrile animals as controls. Following these icv treatments brain temperature was monitored for an additional 60 min.

In a parallel study, the antipyretic drug indomethacin (5 mg/kg, Sigma), was administered sc 30 min prior to endotoxin to observe the effects of indomethacin on AVP-induced antipyresis and on the febrile response of M-AVP-treated rats. Thus, following sc indomethacin, endotoxin was injected icv and the brain temperature was monitored for 150 min. At this point AVP was injected icv and temperatures were followed for an additional 60 min.

To test the effect of M-AVP on the febrile response and drug-induced antipyresis, the antagonist was administered alone or endotoxin was administered 30 min after pre-treatment with indomethacin. As a control, saline was injected icv and 24 h later endotoxin alone or endotoxin plus indomethacin were administered.

All brain temperature responses were expressed as changes from the time of endotoxin administration or changes from 150 min post-endotoxin. Statistical analysis was performed using the Student's t-test or ANOVA and Newman-Keuls test. Statistical significance was considered as $p < 0.05$.

C. Experimental Series 2. AVP at Different Ambient Temperatures

Male Wistar rats (250-350 g) were used in all experiments. Animals were surgically prepared with an icv cannula and glass thermistor as described in Series 1. Some rats were also prepared with a sc catheter as described in Series 1. Housing and other preparatory procedures were also identical to those described in Series 1.

Animals were allowed at least 5 days to recover from surgery and were then divided into febrile and non-febrile groups. These groups were then exposed to cold (4°C), room (25°C) or warm (32°C) ambient temperatures. After recording baseline temperatures for 60 min, the rats in the febrile group received 10 ng E. coli endotoxin icv. The febrile rise in temperature was allowed to develop for 150 min after which 2.5 ng AVP or vehicle control (0.9% sterile saline) was injected icv. All icv injections were administered via 30 gauge injection cannulae attached to PE-20 tubing and a Hamilton microsyringe. AVP and saline injections administered at 150 min post-endotoxin are designated as time 0 in all figures. Temperatures were expressed as changes in brain temperature from that recorded at time 0. Non-febrile animals were treated similarly but did not receive endotoxin. Some

rats received indomethacin sc at time 0 for comparison with AVP-induced antipyresis. Injection sites were confirmed by visual inspection of rats' brains after 5 uL icv of bromophenol blue .

The antipyretic effects of icv AVP were compared between febrile and non-febrile groups using a thermal index (Table I). The thermal index (^{0}C hr) was defined as the area between the control (saline) and experimental (AVP) curves from time 0 to 60 min following icv injection. Thus in Table I the thermal index is calculated by finding the difference between changes in brain temperature during AVP experiments and changes during saline control experiments. In this calculation each rat acted as its own control. This calculation, then, represents the amount that AVP decreased brain temperature compared to saline. Using this analysis a dose response curve was plotted demonstrating the calculated thermal index response versus the dose of AVP administered to the febrile rat. In table II, an antipyretic index was calculated as the difference between changes in brain temperature after AVP or sc indomethacin in non-febrile versus febrile experiments. This calculation represents the amount that AVP or indomethacin decreased temperatures in febrile compared to non-febrile rats. The statistical analysis used to evaluate significance between febrile and non-febrile groups in Table I was the Students t-test. The 2-way ANOVA was used to ascertain statistical significance in data groups shown in Figures 11-17.

D. Experimental Series 3. Thermoregulatory Effector Mechanisms

Male Wistar rats were used in all experiments. Under pentobarbital anaesthesia, animals were surgically prepared with an icv cannula and glass thermistor as previously described in Series 1. Animals used for metabolic rate determination had brain temperatures (Tb) measured via biotelemetry (Mini-mitter Inc.). This allowed on line data

acquisition using an Apple IIe micro-computer. Rats were housed individually in plastic cages (Nalgene) and had free access to food and water. Following recovery from surgery (5-7 days), animals were placed in 3 groups based on the experimental ambient temperature (4, 25 and 32°C). At each ambient temperature, Tb and tail skin temperature (Tsk) or Tb and oxygen consumption were measured. Tsk was measured as an indication of cutaneous vasodilation and therefore of cutaneous heat loss. Yellow Springs surface probes (400 series) were taped to the skin at the base of the tail. Rats placed at 32°C also had evaporative heat loss (EHL) determined from the amount of water absorbed in Drierite dessicant placed on line. At the time of AVP or saline injection pre-weighed Drierite was attached to the effluent air line. After 60 min the dessicant was weighed and the amount of water vapour collected over 60 min was determined and converted to EHL using 0.7 W hr/g H_2O (Schmidt-Neilsen, 1984). As an index of heat production, metabolic rate (M) was calculated from the oxygen consumption, corrected to standard temperature and pressure, and assuming 1.0 L oxygen = 20.1 kJ and a respiratory quotient of 0.82 (Schmidt-Neilsen, 1984). Rats were placed in a sealed plexiglass chamber (4 L) (Figure 4) and breathing grade air (Medigas; 20.9% O_2, 79% N_2 and 0.03% CO_2) flowed through the chamber at 1.0 L/min. Oxygen levels were measured in the air before and after entering the chamber using a Beckman OM-11 oxygen analyzer. Typical values for the differences between in and outgoing air were 0.7%, 1.2% and 0.4% O_2 at ambient temperatures of 25, 4 and 32°C respectively. Sensitivity of the oxygen analyzer was 0.1%. In order to determine baseline values, Tb, Tsk and oxygen consumption were monitored for at least 60 min prior to commencing the experiment. Following this, 10 ng of endotoxin was injected icv and the resulting changes in Tb, Tsk and oxygen consumption were then followed for an additional 150 min. In all graphs time 0 represents the time of AVP or control challenge at 150 min post-endotoxin. For Tb and Tsk the values at 150 min were normalized to 0 and the resultant changes plotted. Statistical analysis was performed using the Student's t-test for paired groups. Significance was considered as p< 0.05.

FIGURE 4

Diagram illustrating the plexiglass chamber and other equipment used for oxygen consumption analysis.
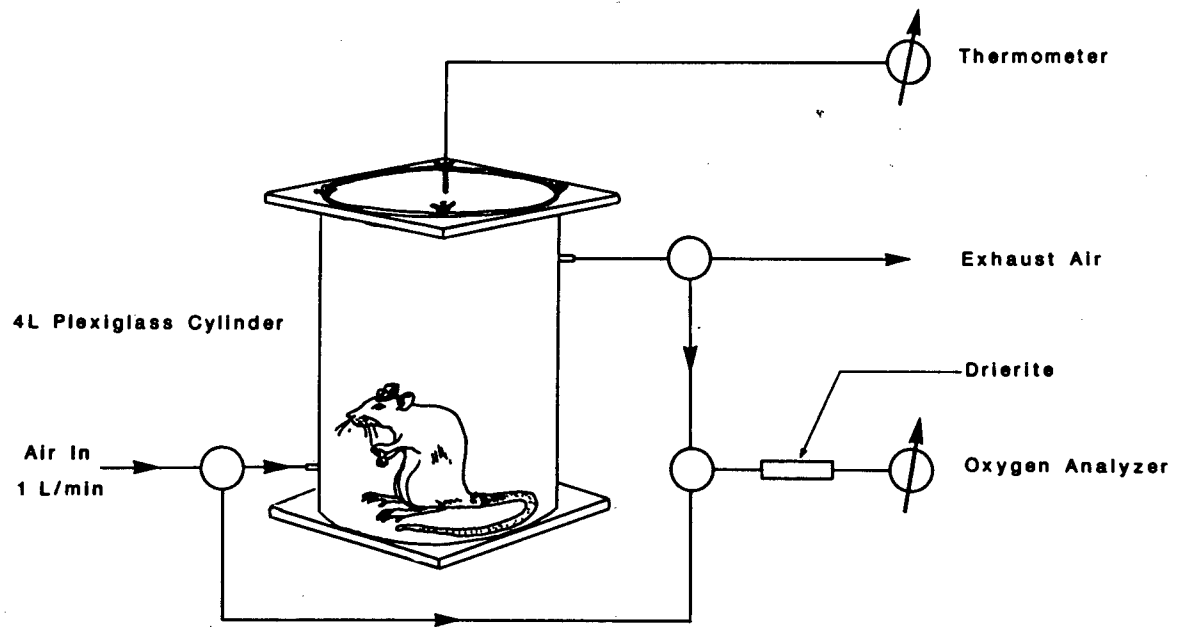
E. Experimental Series 4. AVP in the CSF

Male Wistar rats (280-320 g) were used in all experiments. Rats were anaesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and surgically prepared with an intra-atrial catheter (for intravenous injections) and a CSF withdrawal cannula. A Silastic catheter filled with heparinized saline was passed into the jugular vein and advanced until the tip lay in or near the right atrium. The catheter was then secured with sutures and passed subcutaneously to the top of the skull. The free end was then positioned on to a 20 gauge "U"-tube which was further attached to a short piece of PE-100 tubing. This piece of PE-100 was subsequently plugged after filling the catheter line with heparin (1000 U/mL). After the intravenous catheter was secured the rat was positioned in a stereotaxic frame with the head in a horizontal position. The exposed skull then had 5 holes drilled in order to accommodate jewellers screws. A 6th hole was drilled in the midline 1 mm rostral to the interparietal-occipital suture. The cannula stylet assembly was then inserted free hand with the bevel facing rostrally. The assembly was then slowly advanced along the inner aspect of the occipital bone until the soldered cross-piece prevented further advancement. At this point the cannula tip rested in the cisterna magna. Proper placement was checked by removing the stylet and observing CSF flow out the top of the cannula. The cannula and iv catheter were then secured with applications of dental acrylic. Each rat was individually housed in plastic cages with free access to food and water. A 12:12 light-dark cycle was maintained.

Recovery from surgery usually took 5-7 days. Prior to starting experiments rats were given a 1-2 day acclimation period. This time allowed the animals to become accustomed to the insertion of a rectal probe for body temperature measurement. For CSF collection, the stylet was removed and a pre-calibrated PE-100 line, connected to a 1.0 mL syringe, was attached to the withdrawal cannula. Blood free CSF (100 uL) was slowly

withdrawn and separated into 50 uL aliquots and frozen at -65°C until assayed. This volume was withdrawn 4 times daily at 2.5 h intervals. During sampling the stylet was stored in 70% ethanol and immediately replaced following every sample. Due to the circadian release properties of AVP into the CSF (Mens et al, 1982; Reppert et al, 1983; Schwartz et al, 1983), great care was taken to sample CSF at the same time each day. Rats receiving endotoxin (100 ug/kg, iv) were sampled for CSF in the morning and then injected with endotoxin. CSF was then collected as in the control group. Baseline temperatures were monitored via a rectal probe inserted 6 cm beyond the anus (Yellow Springs) for 60 min prior to iv injection of endotoxin. Temperatures were then recorded every 30 min for the duration of the experiment. Statistical analysis was performed using the Student's t-test for paired groups. Significance was considered as $p < 0.05$.

AVP concentrations in the CSF were measured using a radioimmunoassay with a sensitivity of 0.13 - 0.22 pg/tube. A rabbit anti-AVP antiserum was used at a final concentration of 1:810 000 The assay used 50 uL of anti-AVP antiserum, 50 uL of sample, 250 uL of buffer (0.1 M sodium phosphate, pH 7.2 containing 0.01 M EDTA, 0.05 M NaCl, 0.02% sodium azide, and 0.01% BSA) and 100 uL of iodinated-AVP (New England Nuclear) at 4000 counts per minute (cpm) per tube. The assay was incubated at 4°C for 2 days without label and then 2 days with label. The double antibody technique was used to precipitate the bound label and the pellet was counted in a gamma counter and calculated by log-logit method. Intraassay (within) variability was 8.3% and interassay (between) variability was 10.5% for a 1.04 pg standard. The 50% B/Bo (the ratio of labelled peptide bound in the presence of AVP standard to that bound in the absence of any AVP) was 1.52 pg and the slope of the curve was -1.72. Synthetic AVP added to plasma and acid extracts of the neural lobe of the pituitary show curves which are parallel to synthetic AVP whereas arginine vasotocin (AVT) or oxytocin (OXY) show non-parallel curves. The antibody cross-reacts 17% with AVT, 0.2% with OXY and

<0.01% with all other peptides tested; somatostatin-14, bombesin, cholecystokinin-8, bradykinin, angiotensin II, neurotensin, Leu-enkephalin, thyroid-stimulating hormone releasing hormone and vasoactive intestinal polypeptide.

III. RESULTS

A. Experimental Series 1. AVP Antagonist

The effect of endotoxin (10 ng) administered icv on the brain temperature of the rat is demonstrated in Figure 5. It is evident from this figure that by 150 min post-endotoxin a fever of at least 1.0°C above the baseline has developed. Figure 5 also demonstrates the injection protocol utilized in Experimental Series 1, 2 and 3. That is endotoxin at time 0 followed by AVP, M-AVP or saline 150 min later. In this figure AVP (5 ng) administered icv caused a decrease in the brain temperature of febrile rats (n=8) from 1.05 ± 0.17 to 0.21 ± 0.24°C by 30 min after injection of AVP. This was significantly different from saline-treated controls (n=6). AVP administered icv in non-febrile rats (n=9) at the same dose had no consistent effect on brain temperature indicating that the peptide was antipyretic rather than hypothermic (Figure 6).

In order to assess the effects of the AVP analog, M-AVP, on the antipyretic action of AVP, the analog was injected icv prior to AVP in febrile rats. Figure 7 summarizes the results. It is clear that M-AVP given 2 min or 24 h prior to AVP prevented the antipyresis induced by AVP. By 30 min the maximum change in Tb of the febrile rat induced by AVP alone was -0.85 ± 0.17°C. After pre-treatment with the analog the changes induced at 30 min post-AVP injection were $+0.13 \pm 0.11$°C and $+0.27 \pm 0.10$°C for the 2 min and 24 h pre-treatment groups respectively. Furthermore this abolition of the AVP-induced effect was not merely a hyperthermic negation of the antipyresis since M-AVP alone had no effect on Tb in febrile (n=5) or non-febrile (n=6) rats (Figure 8).

In a second series of experiments, the antipyretic drug, indomethacin, was administered sc in order to evaluate its effects during endogenous AVP blockade with

FIGURE 5

Mean change in brain temperature in rats receiving endotoxin icv at time 0. At 150 min (arrow) febrile animals received either AVP (closed circles, n=8) or saline (open circles, n=6) icv. This figure illustrates the injection protocol utilized in all experiments. Each point denotes mean $\pm$ SEM. * $p < 0.05$, ANOVA and Newman-Keuls test.
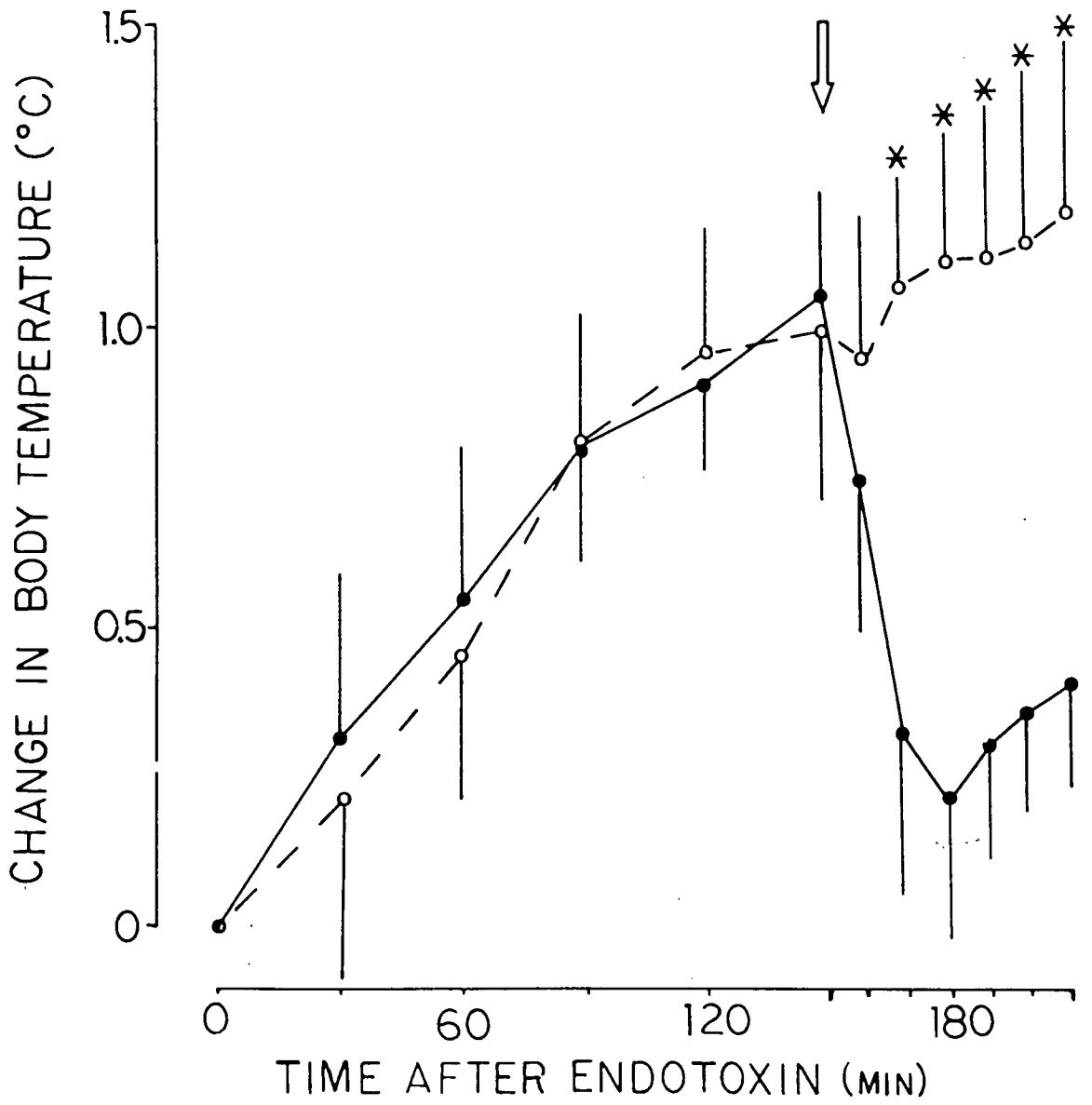
FIGURE 6

Mean change in brain temperature of non-febrile rats receiving AVP (closed circles, n=9) or saline (open circles, n=6) icv. Each point denotes mean $\pm$ SEM. There were no statistical differences between the groups.
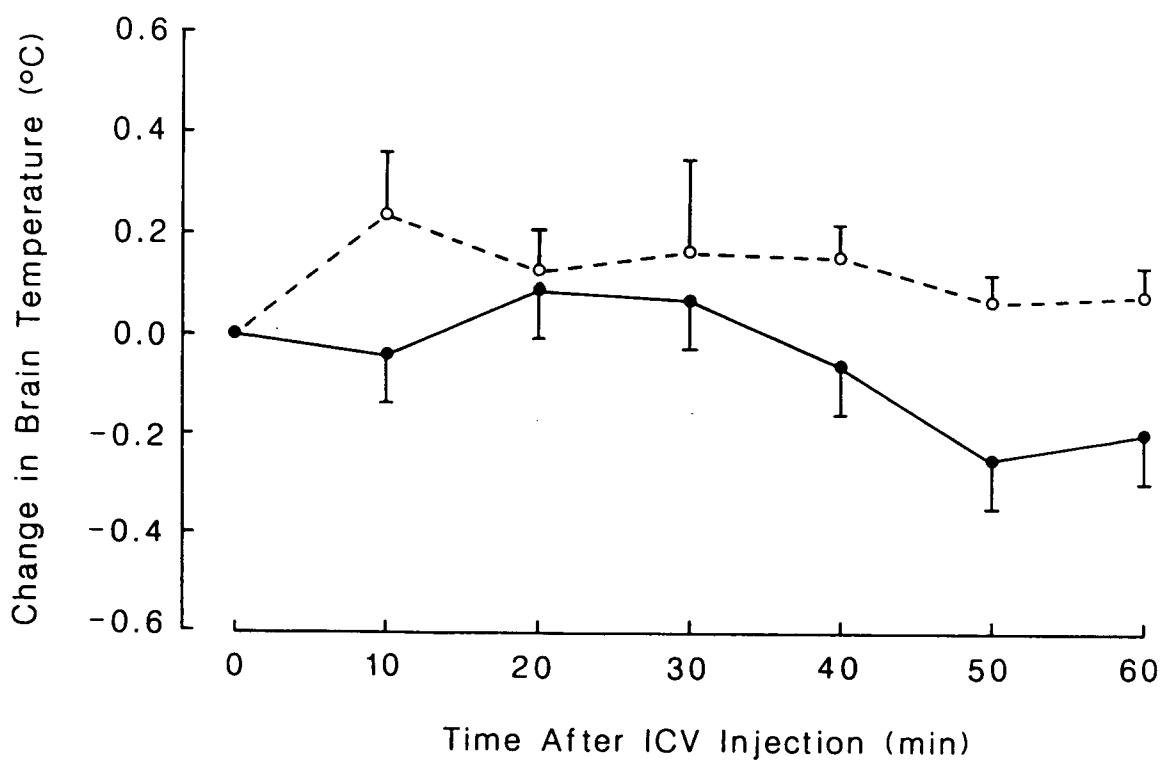
FIGURE 7

Mean change in brain temperature of febrile rats which received the following icv injections 150 min post endotoxin: saline (closed circles, n=8), M-AVP 2 min (open circles, n=5) or M-AVP 24 h (open squares, n=8) prior to AVP icv. * $p<0.05$ ANOVA and Newman-Keuls test. Each point denotes mean $\pm$ SEM.
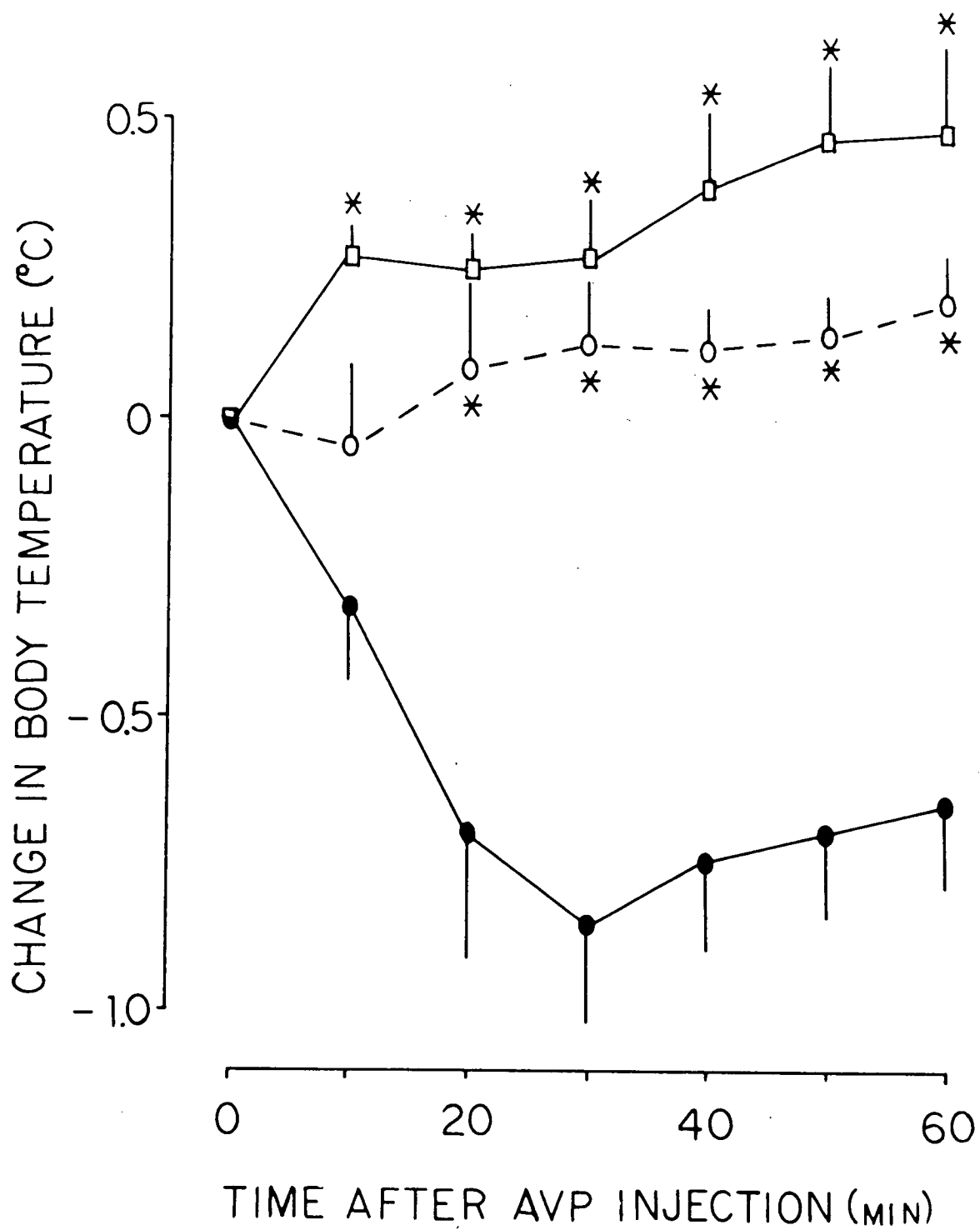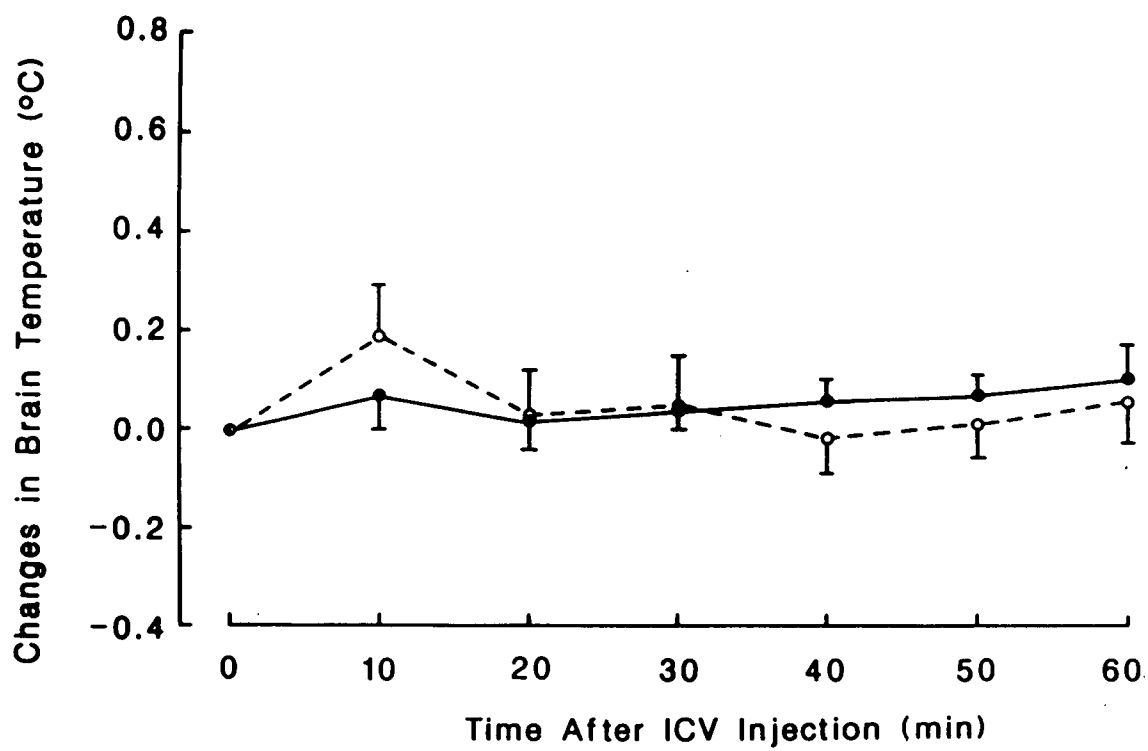
FIGURE 8

Mean change in brain temperature of febrile (closed circles, n = 5) and non-febrile (open circles, n = 6) rats receiving M-AVP icv. Each point denotes mean $\pm$ SEM. There were no statistical differences between the groups.

M-AVP and during AVP-induced antipyresis. Figure 9 compares the results of antipyresis with AVP alone (-0.85 $\pm$ 0.17°C) and that induced by AVP during drug induced-antipyresis with indomethacin (-0.92 $\pm$ 0.32°C). These were not significantly different. When the febrile responses were examined in M-AVP-treated versus saline-treated rats (Figure 10) it was apparent that the mean fever heights were no different despite endogenous AVP blockade. The endotoxin fever (1.46 $\pm$ 0.22°C) of saline controls was not significantly different than that after 24 h pre-treatment with the antagonist (1.63 $\pm$ 0.22°C). Indomethacin caused significant antipyresis, lowering fever about 50% to 0.73 $\pm$ 0.12°C but pre-treatment with the antagonist did not alter the degree of antipyresis and resulted in fevers with a magnitude of 0.64 $\pm$ 0.25°C (Figure 10).

B. Experimental Series 2. AVP at Different Ambient Temperatures

In order to establish a dose-effect relationship a series of febrile rats (n = 49) were given doses of AVP ranging from 0.5 ng to 5.0 ng following the usual protocol (see Figure 5). The Tb responses were then converted to the thermal index ($^{\circ}$C hr) and plotted against the dose of AVP (Figure 11). It should be noted that in many rats AVP injections that were higher then 5.0 ng often produced side effects involving severe motor disturbances and in a few cases, death. These side effects precluded the continuation of the dose response curve. Figure 11 does demonstrate however, a dose-dependent antipyretic effect elicited by AVP over a small dose range. Based on this data a lower dose (2.5 ng) of AVP was chosen for the remaining studies since it was below the threshold for motor disturbances but still markedly antipyretic.

AVP (2.5 ng) microinjected icv into non-febrile rats at 25°C had no significant effect on Tb compared to saline controls (Figure 12A). In contrast icv AVP administered to

FIGURE 9

Mean change in brain temperature of febrile rats undergoing antipyresis induced by AVP alone (closed circles, n=8) or indomethacin sc plus AVP icv (open circles, n=6). Each point denotes mean $\pm$ SEM. There were no statistical differences between the groups.
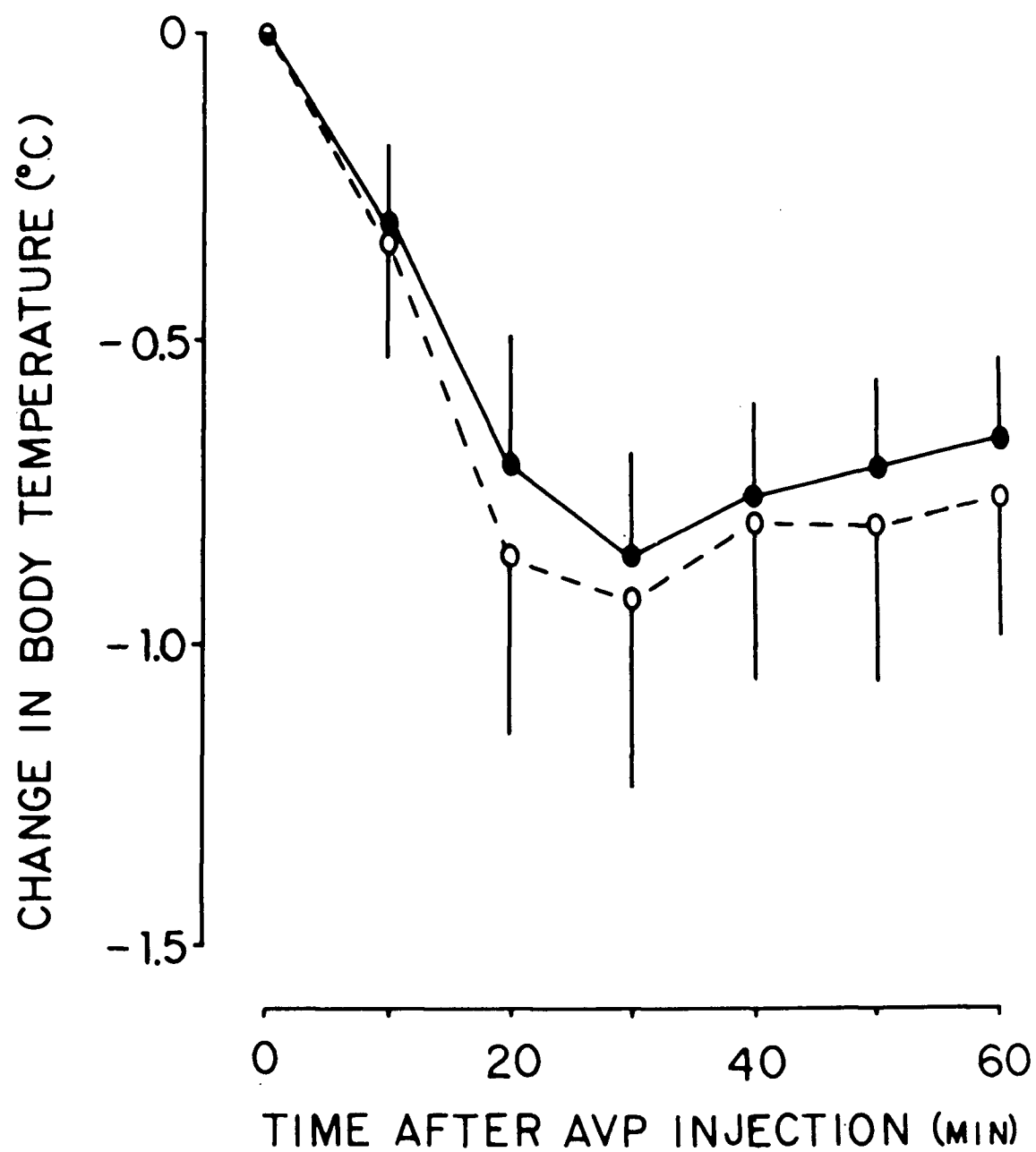
FIGURE 10

Comparison of mean fever responses and indomethacin-induced antipyresis in rats pre-treated with M-AVP (hatched bars) or saline (open bars). M-AVP group: endotoxin + indomethacin, n=6; endotoxin only, n=6. Saline group: endotoxin + indomethacin, n=8; endotoxin only, n=6. Values are mean $\pm$ SEM. There were no statistical differences between the pre-treatment groups.

FIGURE 11
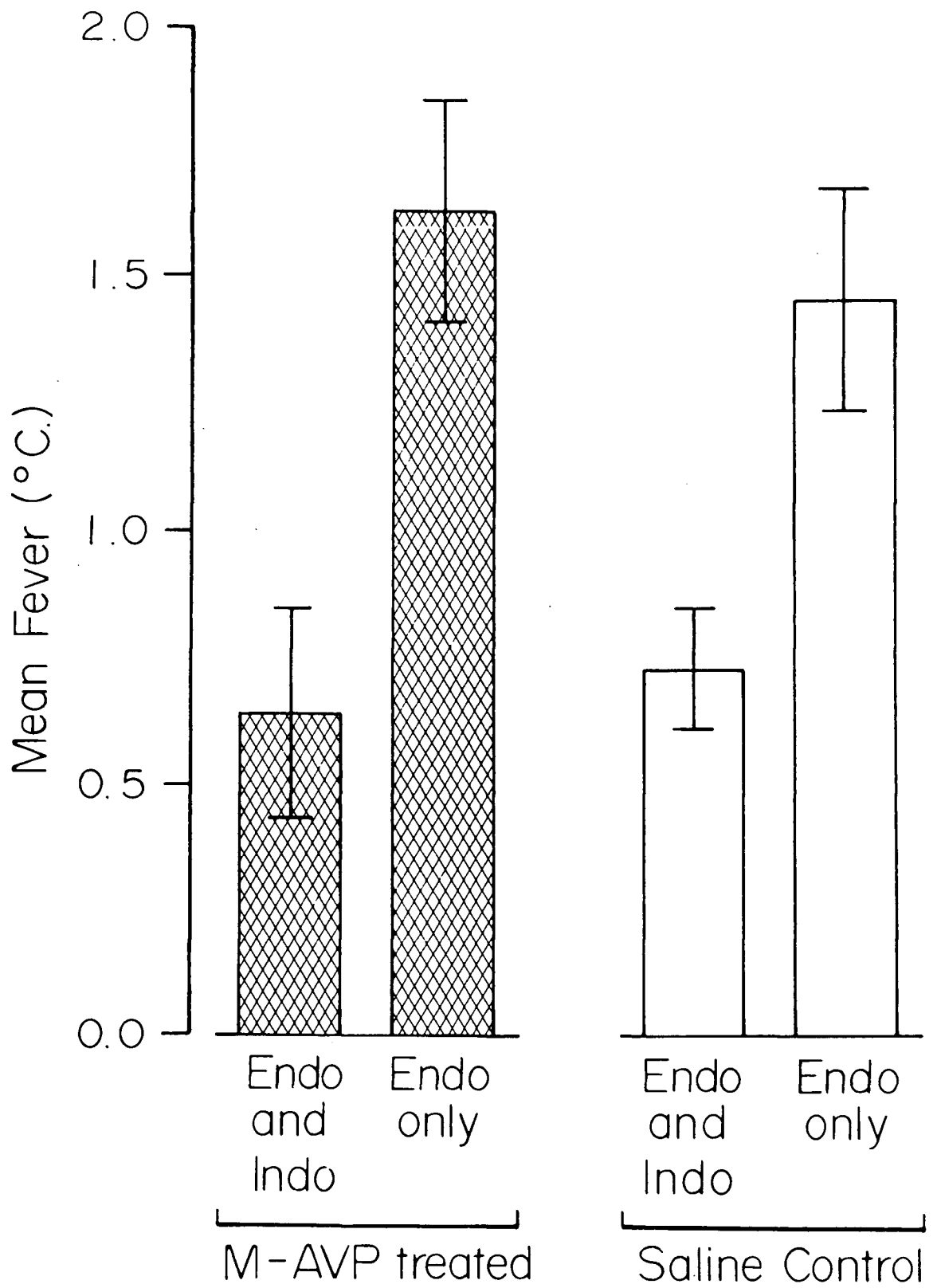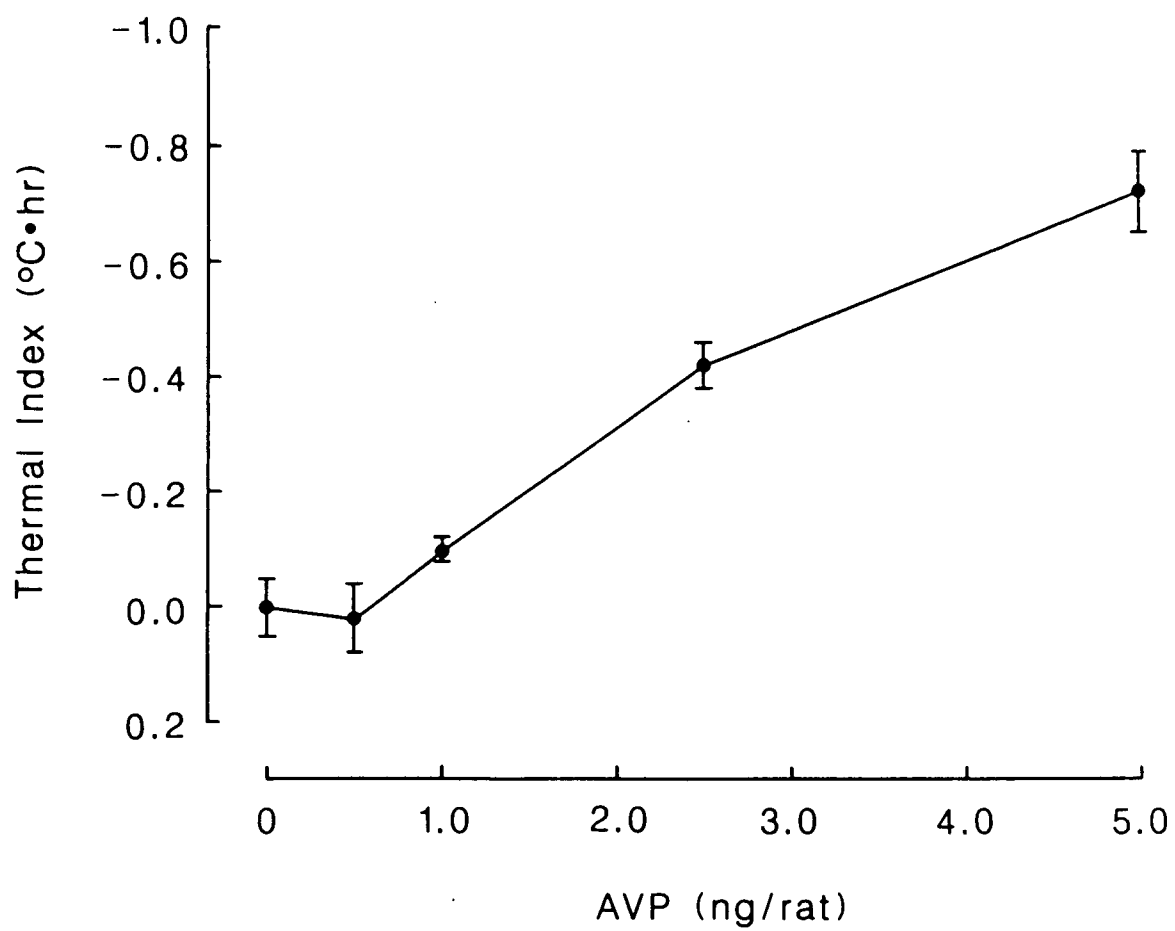
Dose response curve to AVP icv in febrile rats (n=49). The thermal index was calculated as the area between the temperature time curves, in ^{o}C hr, between AVP and saline treated rats. Each point denotes mean $\pm$ SEM.

febrile rats (mean fever 1.2 ± 0.1^{o}C) caused a marked antipyresis (Figure 12B). There was no decrease in Tb in febrile animals receiving saline icv (mean fever 1.4 ± 0.1^{o}C).

In non-febrile rats exposed to warm ambient temperatures (32°C), icv AVP had minimal effects on Tb (Figure 13A). Saline injections in non-febrile warm-exposed rats caused a slight increase in temperature towards the end of the exposure. AVP induced an antipyresis in febrile rats at 32°C (mean fever 2.0 ± 0.1^{o}C) relative to saline-treated febrile rats (mean fever 2.1 ± 0.2^{o}C) (Figure 13B).

During acute cold exposure (4°C) icv saline had no effect on brain temperature in either non-febrile (Figure 14A) or febrile (mean fever 0.9 ± 0.2^{o}C) (Figure 14B) animals whereas icv AVP caused a statistically significant decrease in Tb of febrile animals (mean fever 1.1 ± 0.1^{o}C) but not in non-febrile animals. Temperature of non-febrile animals decreased after AVP but this did not reach significance compared to saline injected controls.

Non-febrile rats receiving indomethacin had no significant change in Tb at 25°C (Figure 15). However, indomethacin decreased Tb in febrile animals at this ambient temperature. At 32°C, indomethacin effectively decreased Tb of febrile rats while not affecting non-febrile animals (Figure 16) although this trend did not reach statistical significance. During cold exposure (4°C), however, indomethacin decreased brain temperatures of both non-febrile and febrile rats (Figure 17).

The data are summarized in Tables I and II. Table I compares the thermal index for non-febrile and febrile animals at the 3 ambient temperatures. It is clear that AVP was

FIGURE 12

Change in brain temperature in response to icv AVP (solid circles) or saline (open circles) at an ambient temperature of $25^{\circ}C$. A. non-febrile rats (n=6) ANOVA, AVP vs saline F = 0.1, NS; B. febrile rats (n=5) ANOVA, AVP vs saline F = 24.3, p<0.05. Each point denotes mean $\pm$ SEM.

A $T_A = 25°C$ Non-febrile

B $T_A = 25°C$ Febrile
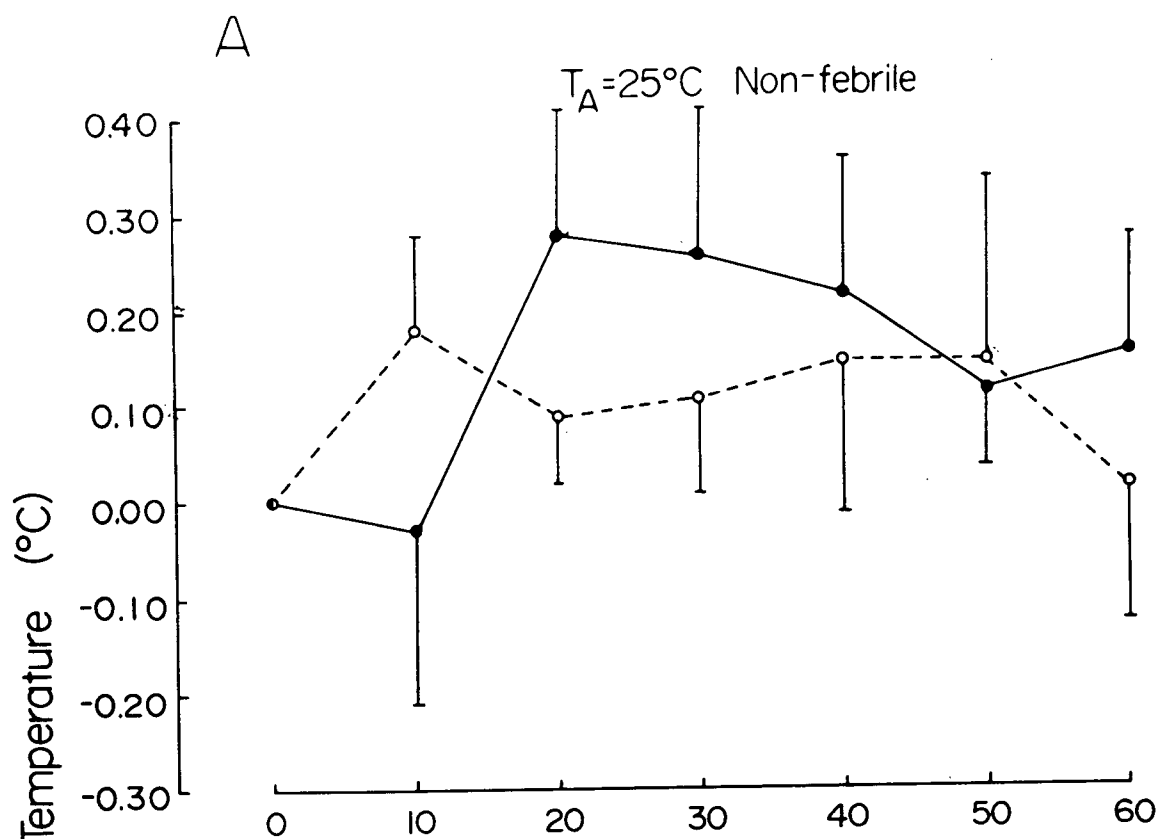
FIGURE 13

Change in brain temperature in response to icv AVP (solid circles) or saline (open circles) at an ambient temperature of $32^{\circ}C$. A. non-febrile rats (n=5) ANOVA, AVP vs saline F = 7.5, NS; B. febrile rats (n=12) ANOVA, AVP vs saline F = 13.9, p<0.05. Each point denotes mean $\pm$ SEM.

A. $T_A = 32°$ C Non-febrile

B. $T_A = 32°$ C Febrile
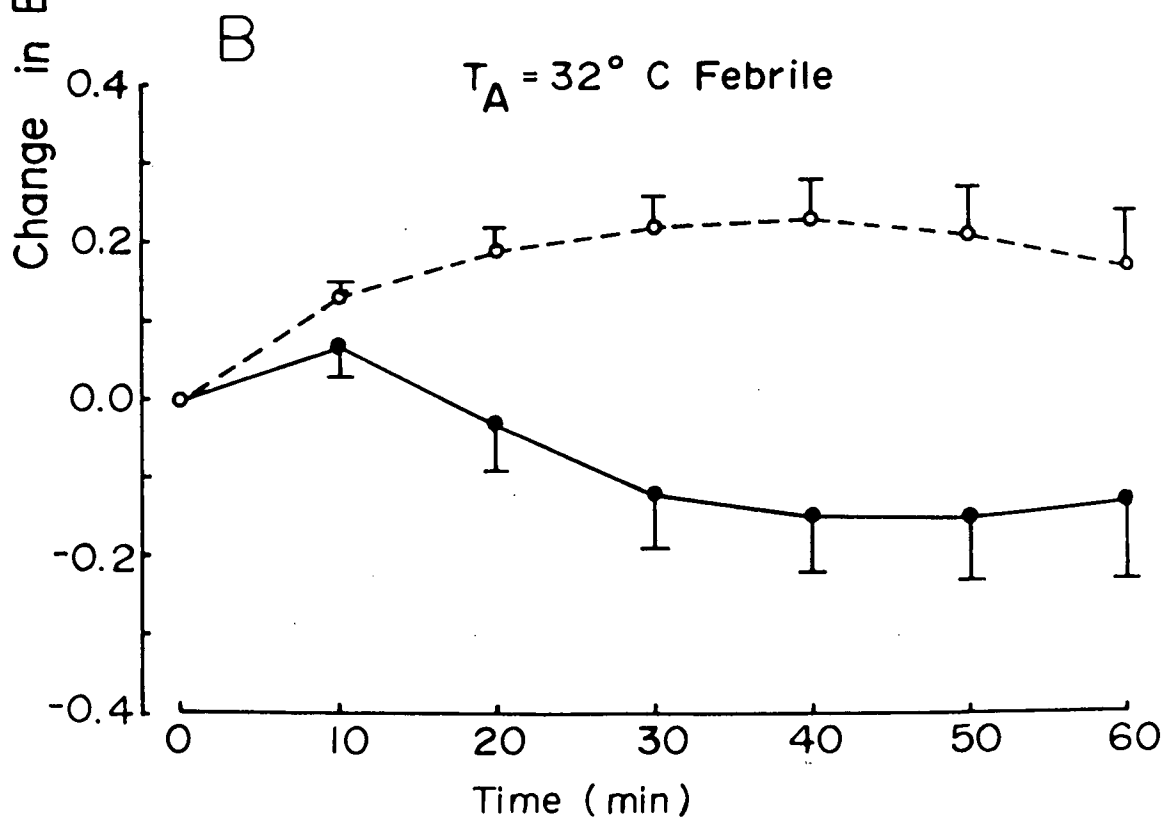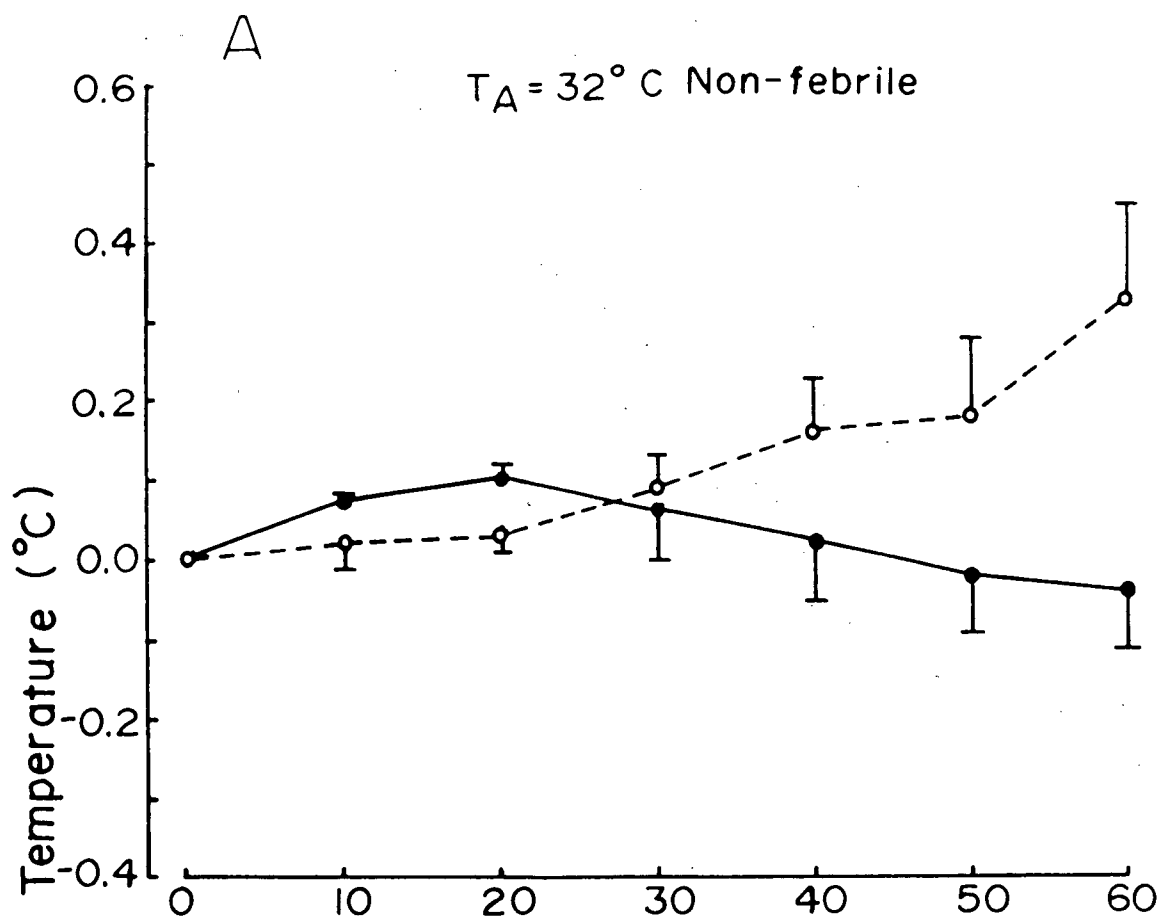
FIGURE 14

   Change in brain temperature in response to icv AVP (solid circles) or saline (open cicles) at an ambient temperature of $4^{\circ}C$. A. non-febrile rats (n=8) ANOVA, AVP vs saline F = 1.7, NS; B. febrile rats (n=7) ANOVA, AVP vs saline F = 30.6, p<0.05. Each point denotes mean $\pm$ SEM.
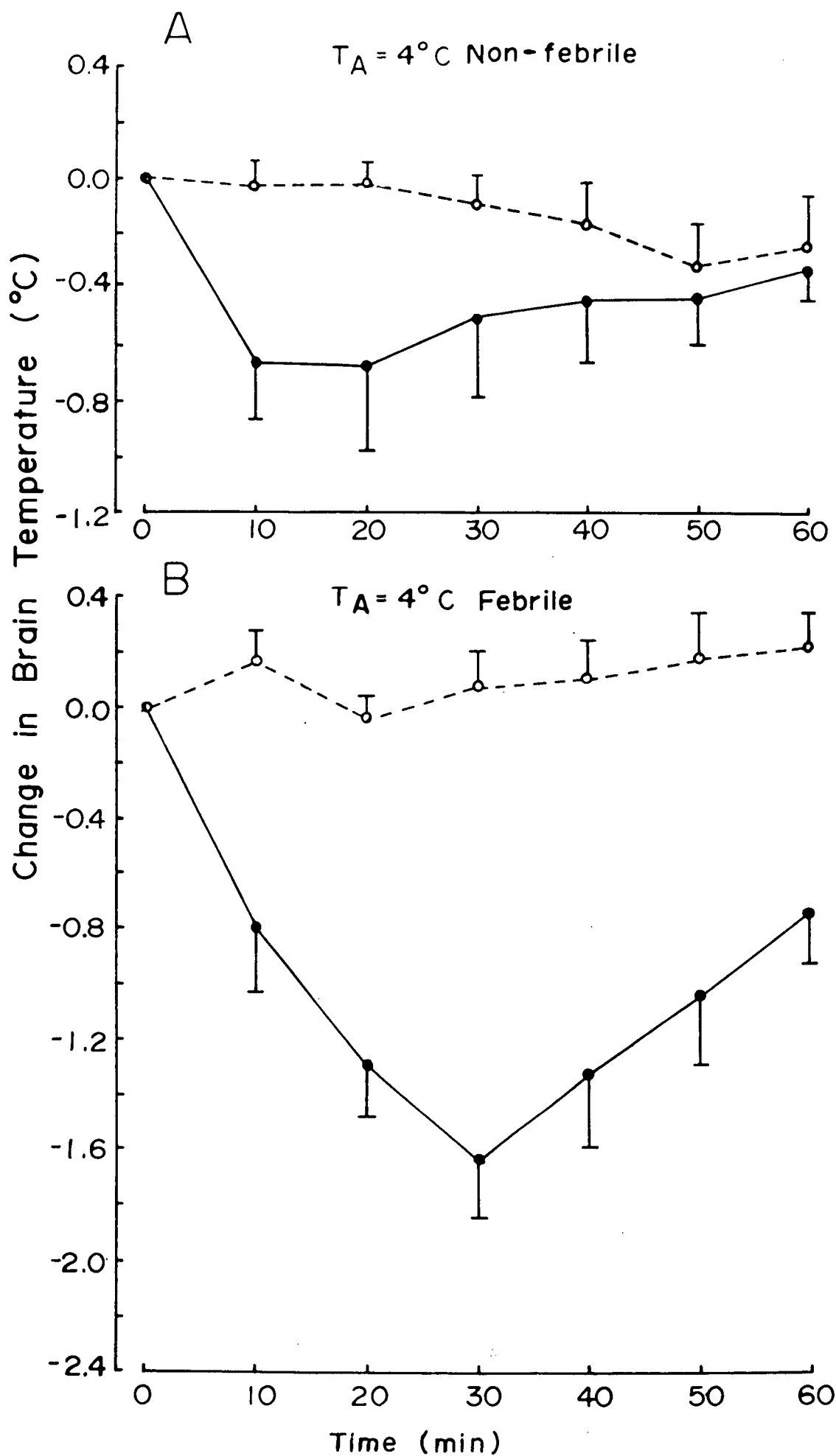
FIGURE 15
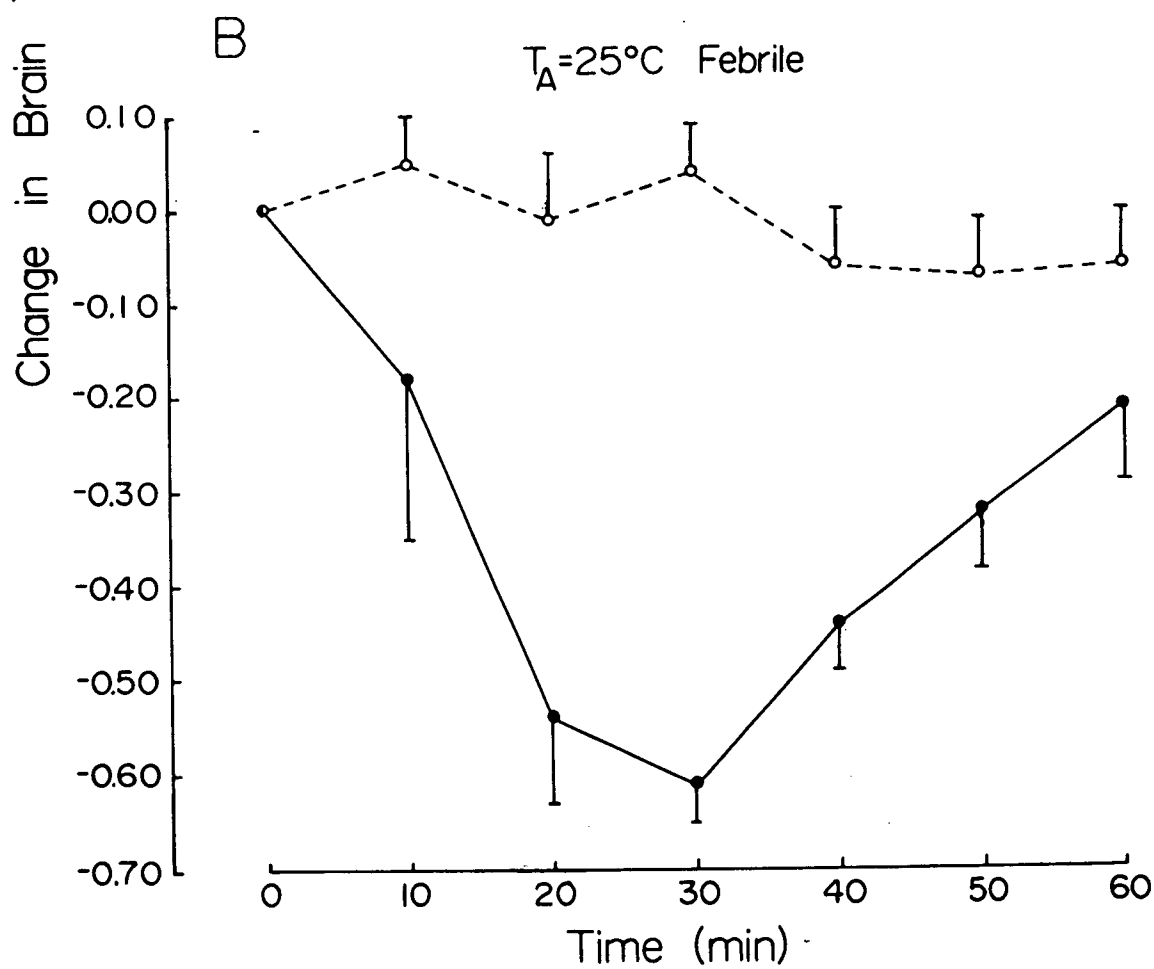
Change in brain temperature in response to sc indomethacin (5 mg/kg) at 25°C. Febrile rats (solid circles, n=6); Non-febrile rats (open circles, n=5). ANOVA, febrile vs non-febrile F = 18.1, p<0.05. Each point denotes mean $\pm$ SEM.
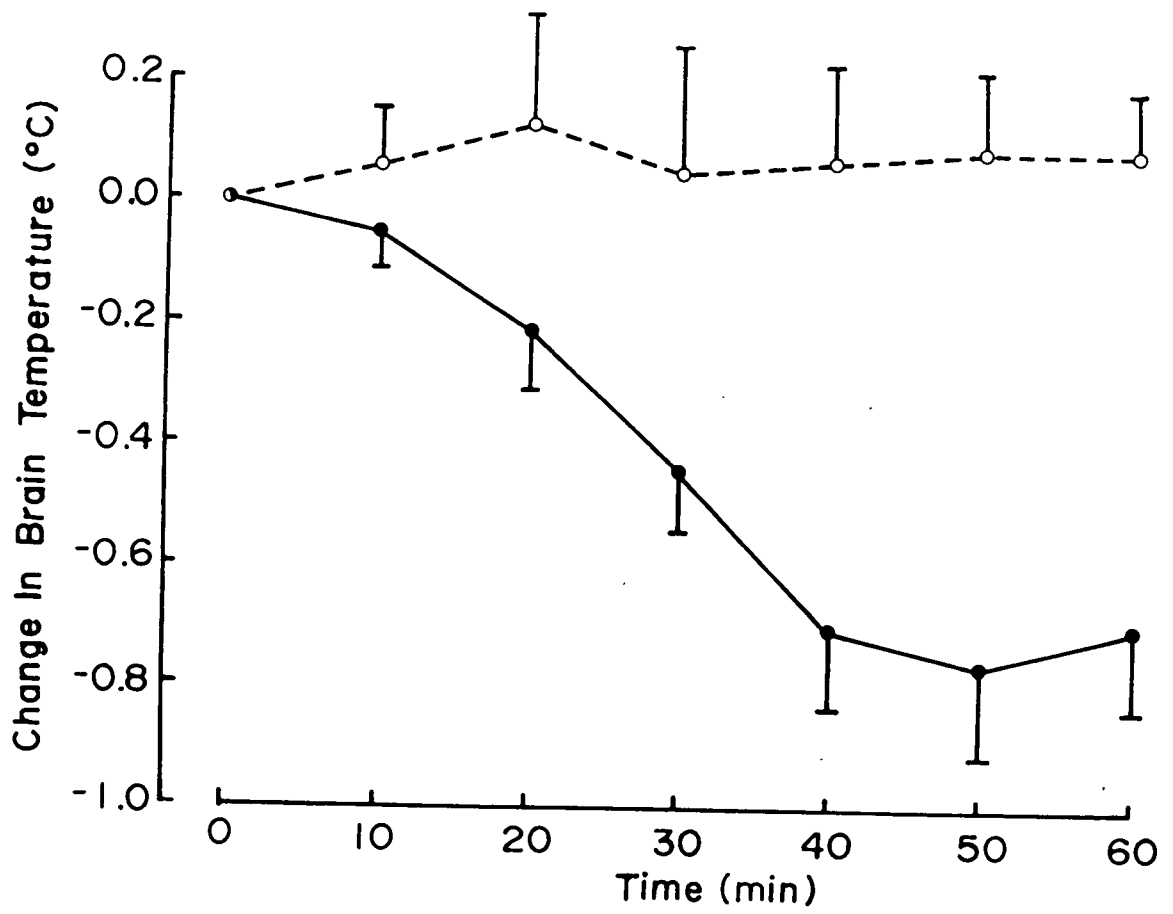
FIGURE 16

Change in brain temperature in response to sc indomethacin (5 mg/kg) at $32^{O}C$. Febrile rats (solid circles, n=6); Non-febrile rats (open circles, n=6). ANOVA, febrile vs non-febrile F = 2.6, NS. Each point denotes mean $\pm$ SEM.
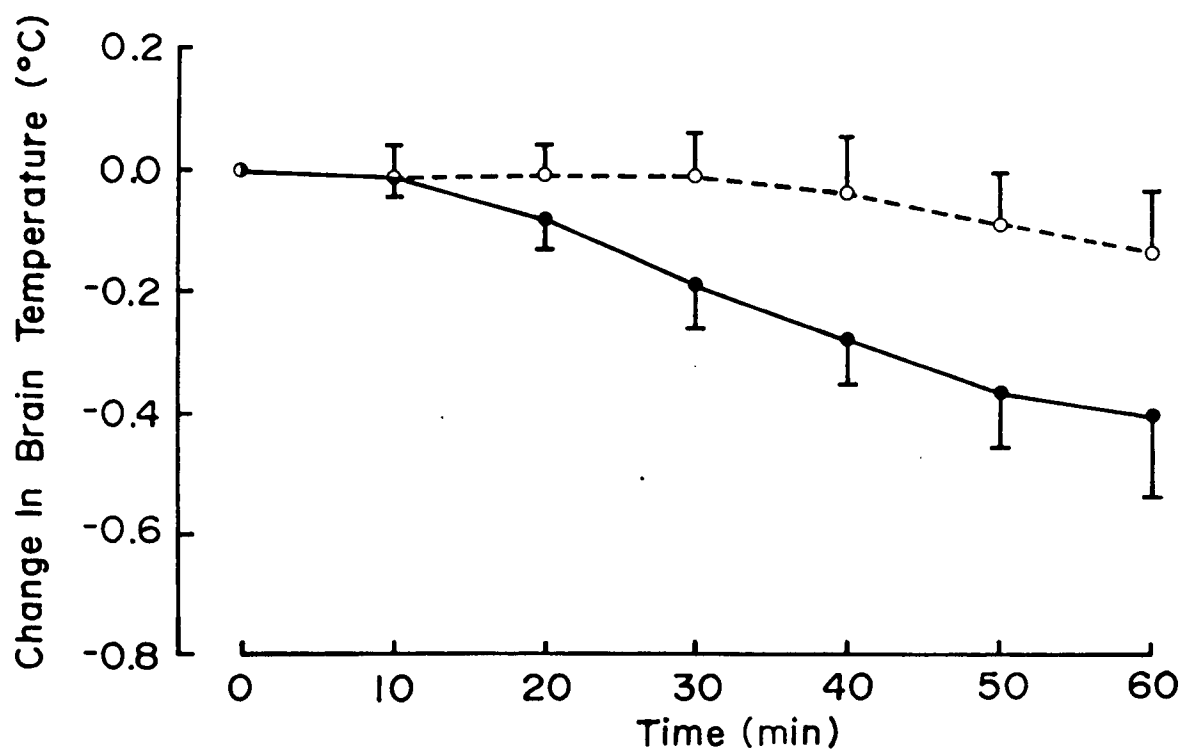
FIGURE 17

Change in brain temperature in response to sc indomethacin (5 mg/kg) at 4°C. Febrile rats (solid circles, n=7); Non-febrile rats (open circles, n=7). ANOVA, febrile vs non-febrile F = 7.4, p<0.05. Each point denotes mean $\pm$ SEM.
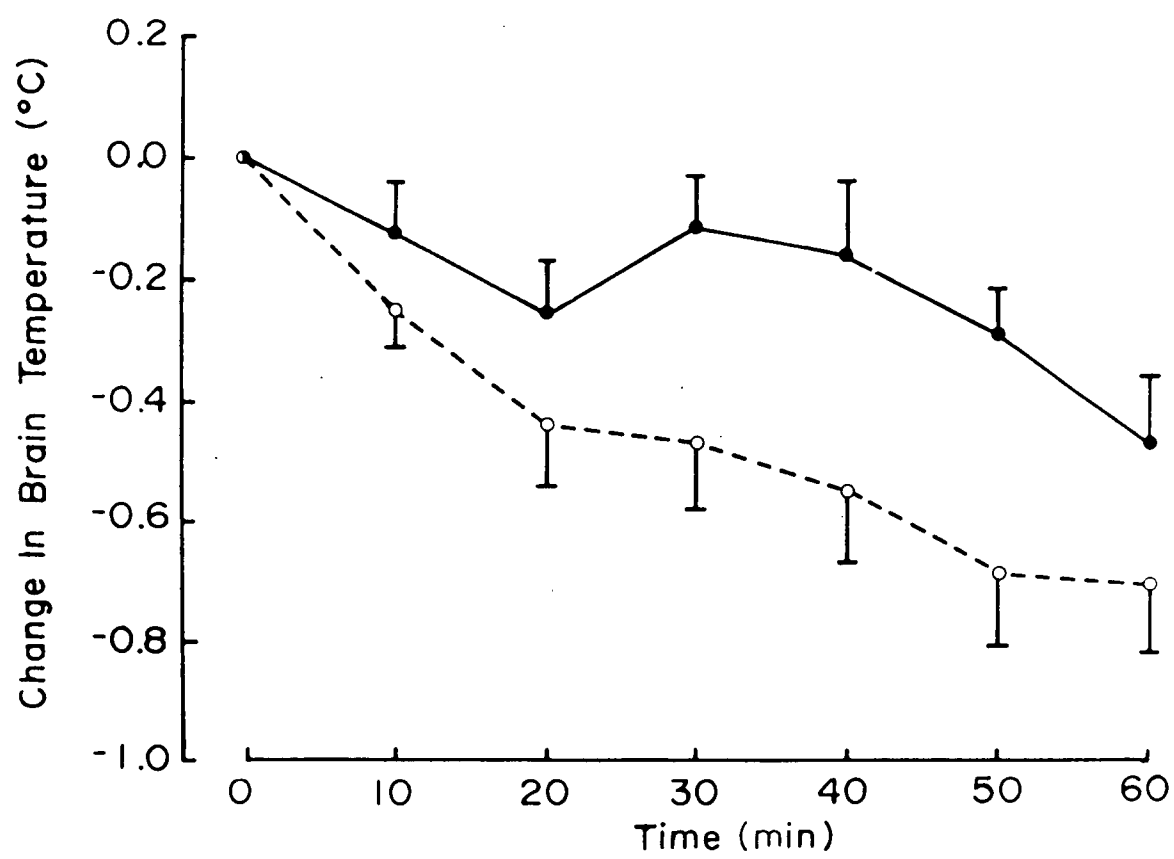
Table I. Comparison of thermal indices in response to icv AVP in febrile
and non-febrile rats.

| Ambient Temp. (°C)	Condition	N	Fever Height (°C)	Thermal Index (°C hr)	
25	Non-febrile	6	-	0.05 ± 0.15	
	Febrile	5	1.24 ± 0.14	-0.36 ± 0.08	*
32	Non-febrile	5	-	-0.08 ± 0.04	
	Febrile	12	2.02 ± 0.12	-0.28 ± 0.07	*
4	Non-febrile	8	-	-0.48 ± 0.27	
	Febrile	7	1.14 ± 0.17	-1.17 ± 0.19	*

Significant difference between febrile and non-febrile groups; Unpaired
t-test: * $p < 0.05$

Table II. Coomparison of antipyresis induced by AVP and indomethacin in
febrile rats.

Ambient Temp. (°C)	Antipyretic Index (°C hr)	
	AVP	Indomethacin
25	-0.34 ± 0.10	-0.59 ± 0.14
32	-0.23 ± 0.08	-0.12 ± 0.10
4	-0.78 ± 0.32	0.29 ± 0.10

more effective at reducing brain temperatures in febrile than in non-febrile rats at all 3 ambient temperatures despite causing some decrease in temperature of non-febrile rats at $4^{\circ}C$.

The antipyretic index calculated for Table II illustrates that at ambient temperatures of $25^{\circ}C$ and $32^{\circ}C$, AVP and indomethacin induced similar degrees of antipyresis. Despite the observations that both AVP and indomethacin decreased brain temperatures of non-febrile rats at $4^{\circ}C$, AVP remained more effective at reducing febrile than non-febrile temperatures whereas this did not hold true for indomethacin.

C. Experimental Series 3. Thermoregulatory Effector Mechanisms.

Table III shows the mean baseline values for all measured variables in the experimental and control groups at each ambient temperature. The effect of ambient temperature alone on these values is apparent and there are no differences between control or AVP-treated groups. Tb was slightly higher in the warm and lower in the cold. M was altered as expected being approximately double in the cold compared to room temperature and half that of room temperature, in the warm. Tsk indicated vasodilation at $32^{\circ}C$ and vasoconstriction at $4^{\circ}C$.

In Table IV, the effects of endotoxin icv on each of these variables is presented. While the maximum fever heights generally occurred by 150 min post-endotoxin, the maximum changes in M or Tsk during fever development were variable and occurred transiently between 60 to 120 min after endotoxin. The trend towards tail skin vasoconstriction and increased M during fever development is expected in view of the

Table III. Pre-endotoxin values for Tb, Tsk and M in experimental and control groups for each ambient temperature. Values are expressed as mean ± SEM. Numbers in parentheses denote the N for each group. Tb = brain temperature, Tsk = tail skin temperature, M = metabolic rate.

	AVP			SALINE	
4°	25°	32°	4°	25°	32°

Tb ($^{\circ}$C)

35.6±0.2	36.4±0.2	37.4±0.3	35.6±0.1	36.0±0.1	37.3±0.3
(13)	(11)	(11)	(13)	(11)	(11)

M (W/kg)

12.2±0.7	7.3±0.4	3.7±0.4	11.4±1.0	7.5±0.7	3.7±0.4
(5)	(5)	(5)	(5)	(5)	(5)

Tsk ($^{\circ}$C)

13.3±1.0	28.1±0.6	35.9±0.2	13.4±0.8	27.9±0.6	34.6±0.4
(8)	(6)	(6)	(8)	(6)	(6)

Table IV. Effect of endotoxin on T_b, T_{sk} and M in experimental and control groups for each ambient temperature. Values are expressed as the mean change $\pm$ SEM for the maximum recorded measurement during 150 min post-endotoxin. Numbers in parentheses denote N for each group.

| | AVP | | | SALINE | |
4°	25°	32°	4°	25°	32°
T_b ($^{\circ}$C)					
1.2+0.15	1.2+0.1	0.9+0.1	1.4+0.1	1.8+0.2	1.2+0.1
(13)	(11)	(11)	(13)	(11)	(11)
M (W/kg)					
2.3+0.6	1.9+0.4	0.5+0.1	3.5+0.5	3.3+0.4	0.4+0.3
(5)	(5)	(5)	(5)	(5)	(5)
T_{sk} ($^{\circ}$C)					
-3.8+0.6	-1.3+0.5	-1.3+0.2	-2.4+0.8	-1.1+0.3	-1.4+0.4
(8)	(6)	(6)	(8)	(6)	(6)

increase in Tb and is in agreement with previous reports (Splawinski et al, 1977; Szekely et al, 1979; Stitt et al, 1985). The relatively modest changes that were measured reflect the small increases in Tb (0.9 to 1.8°C) and the transient nature of endotoxin fever.

The effect of icv AVP on the Tb of febrile animals at 25, 4 and 32°C was similar to that seen in Series 2 (Figure 18A, 19A, 20A). At 25°C AVP administration induced an intense and rapid tail skin vasodilation that paralleled the fall in Tb (Figure 18B). This vasodilation was maximal (6.1 $\pm$ 0.6°C) 10 min after AVP and was indistinguishable from controls by 30 min. The time course and degree of vasodilation is consistent with the idea that the decrease in Tb is caused by this vasodilation. Heat production at 25°C (Figure 18C), however, was not consistently affected by AVP treatment and was not significantly different from saline-treated controls.

At 4°C a robust antipyresis was observed following icv AVP (Figure 19A). This antipyresis was maximal (-1.3 $\pm$ 0.2°C) 20 min after the AVP treatment and was accompanied by cutaneous vasoconstriction (Figure 19B) throughout the 60 min measurement. Vasoconstriction was maximum (-5.8 $\pm$ 1.5°C) 50 min after AVP. Analysis of M at 4°C (Figure 19C) showed that icv administration of AVP induced a precipitous and significant reduction in M which reached a minimum value of 7.5 $\pm$ 0.5 W/kg between 5 and 15 min following injection of the peptide. This inhibition of heat production lasted 15 min and the degree and time course of the inhibition was consistent with the idea that it was the cause of the observed antipyretic effect in the cold.

At 32°C there was a small but significant decrease in Tb following icv AVP (Figure 20A) which could not be attributed to changes in either Tsk (Figure 20B) or M (Figure 20C). When no measurable differences were obtained between Tsk and M, EHL was

FIGURE 18

The effects of icv AVP (closed circles) and saline (open circles) on (A) changes in brain temperature (n=11), (B) changes in tail skin temperature (n=6) and (C) metabolic rate (n=5) in febrile rats at 25°C. Each point denotes mean $\pm$ SEM. * $p<0.05$, paired t-test.

A $T_A = 25°C$

B

C

Time After ICV Injection (min)

FIGURE 19

The effects of icv AVP (closed circles) and saline (open circles) on (A) changes in brain temperature (n=13), (B) changes in tail skin temperature (n=8) and (C) metabolic rate (n=5) in febrile rats at 4^OC. Each point denotes mean $\pm$ SEM. * $p<0.05$, paried t-test.
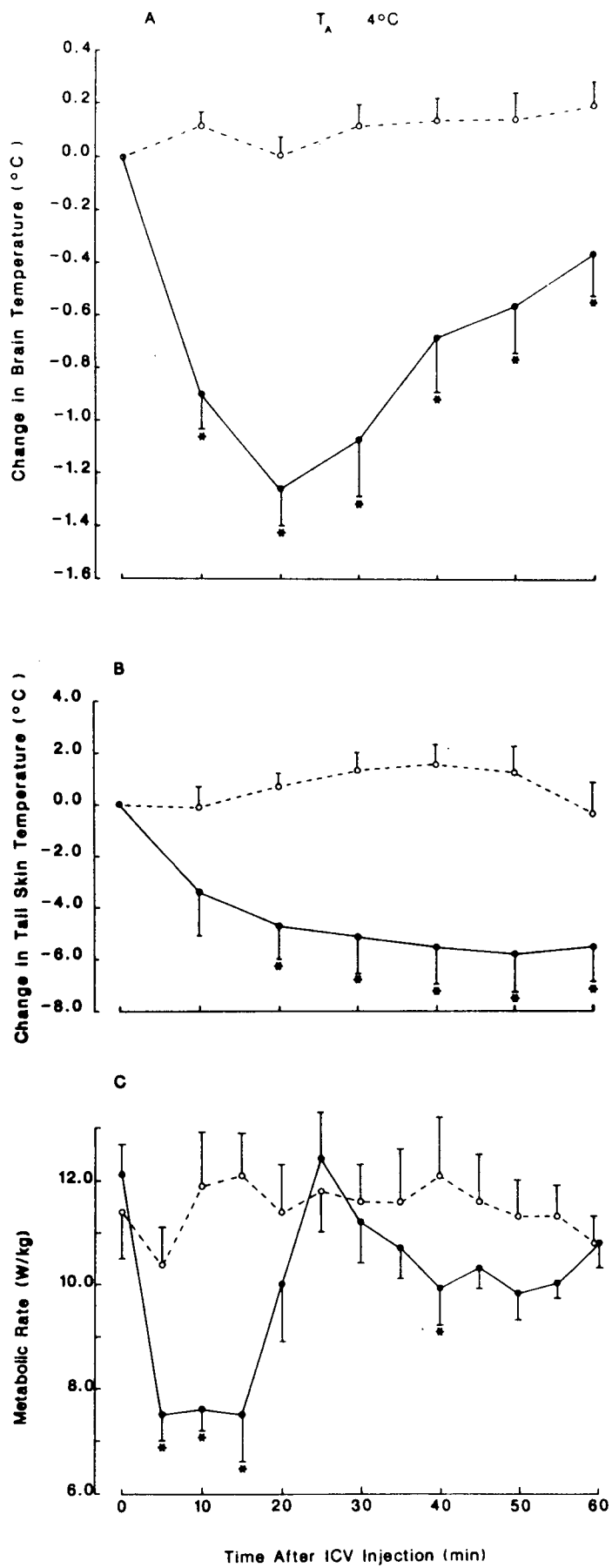
FIGURE 20

The effects of icv AVP (closed circles) and saline (open circles) on (A) changes in brain temperature (n=11), (B) changes in tail skin temperature (n=8) and (C) metabolic rate (n=5) in febrile rats at 32°C. Each point denotes mean $\pm$ SEM. * p<0.05, paired t-test.
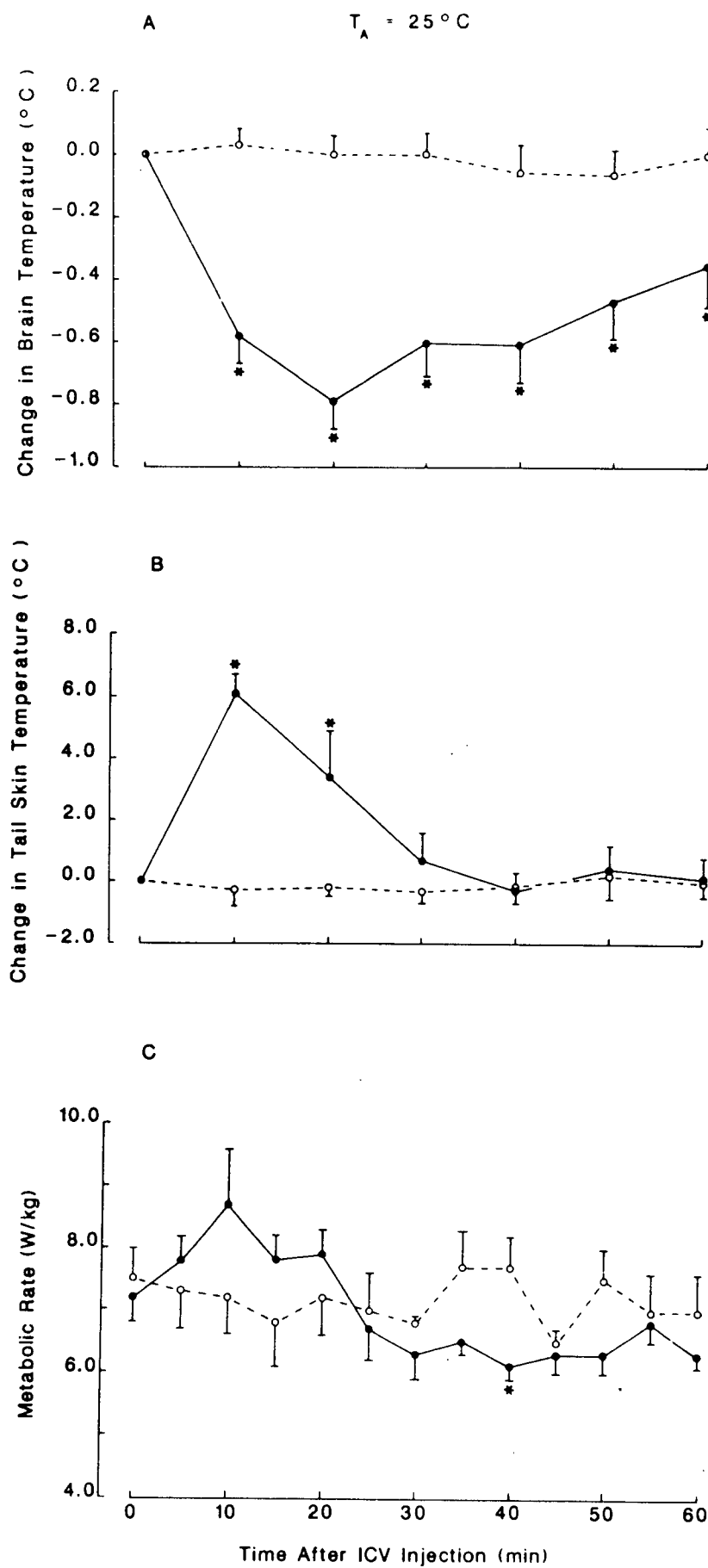
Metabolic Rate (W/kg)

Changes in tail skin temperature (°C)

Change in Brain Temperature (°C)

$T_A = 32°C$
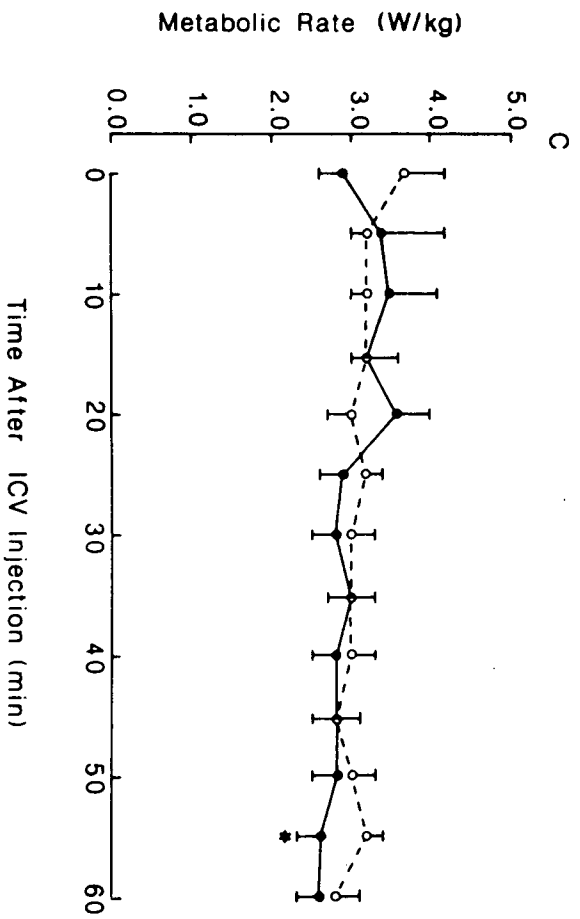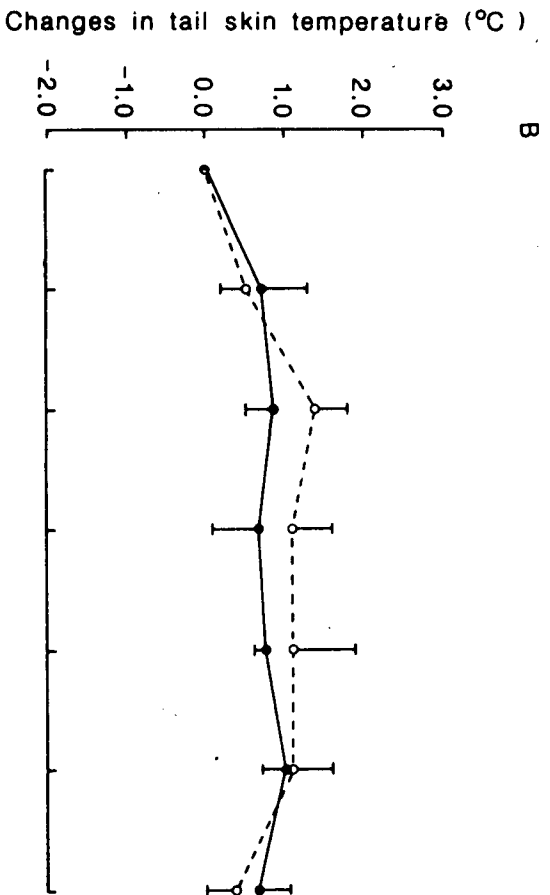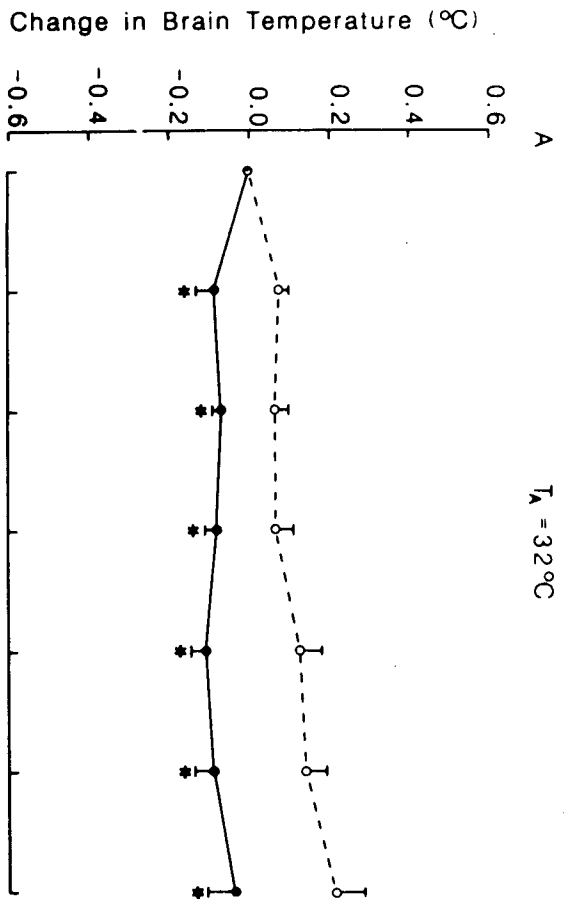
Time After ICV Injection (min)

measured. In AVP-treated rats EHL was 0.39 $\pm$ 0.09 W/kg compared to 0.44 $\pm$ 0.11 W/kg in saline-treated animals. Again there were no significant changes in EHL to explain the observed Tb effect.

D. Experimental Series 4. AVP Levels in the CSF

Rats tolerated CSF sampling without apparent discomfort. Following recovery from surgery free CSF flow could be established in approximately 60% of the animals and CSF could be sampled 4 times daily every 2.5 h for up to 2 weeks. In non-febrile, conscious and unrestrained rats (n=6) AVP in the CSF was measurable and demonstrated a daily rhythm (Figure 21). CSF AVP was low in the morning (0800 to 0900 h) with a mean value of 5.1 $\pm$ 0.7 pg/mL. The AVP concentrations reached a peak between 1130 and 1400 h (7.9 $\pm$ 0.6 pg/mL) and were back to morning levels by late afternoon (1600 to 1730 h) (5.2 $\pm$ 0.4 pg/mL).

The intravenous administration of endotoxin resulted in a typical biphasic temperature response which peaked (mean change 1.8 $\pm$ 0.15^{o}C) by 360 min post-endotoxin (Figure 22). The mean pre-endotoxin baseline temperatures were 36.8 $\pm$ 0.07^{o}C (n=6). The CSF AVP response to iv endotoxin is summarized in Figure 23. Although the peak AVP release has shifted, a point to point analysis revealed that there were no statistical differences in the CSF AVP release pattern in febrile versus non-febrile rats.

FIGURE 21

The cerebrospinal fluid (CSF) AVP release profile in non-febrile rats (n=6). Time 0 was always between 0800 and 0900 h . Values are expressed as mean $\pm$ SEM.
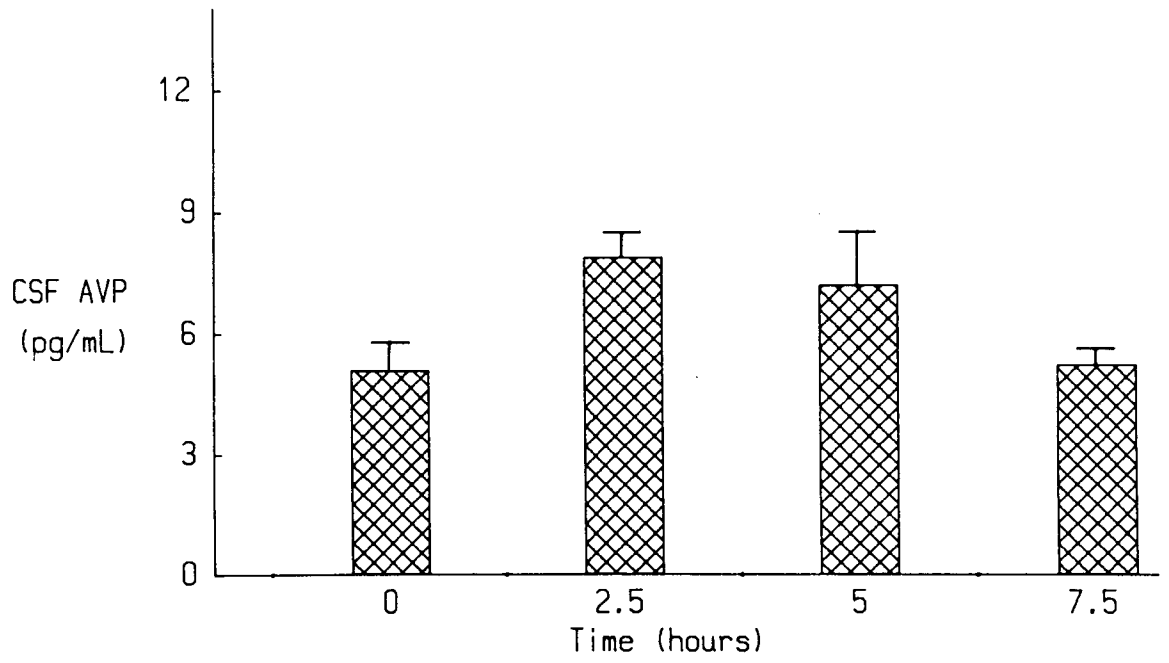
FIGURE 22

Changes in rectal temperature in rats receiving iv endotoxin (100 ug/kg,n=6). The mean pre-endotoxin baseline temperatures were 36.8 $\pm$ 0.07^{O}C. Each point denotes mean $\pm$ SEM.
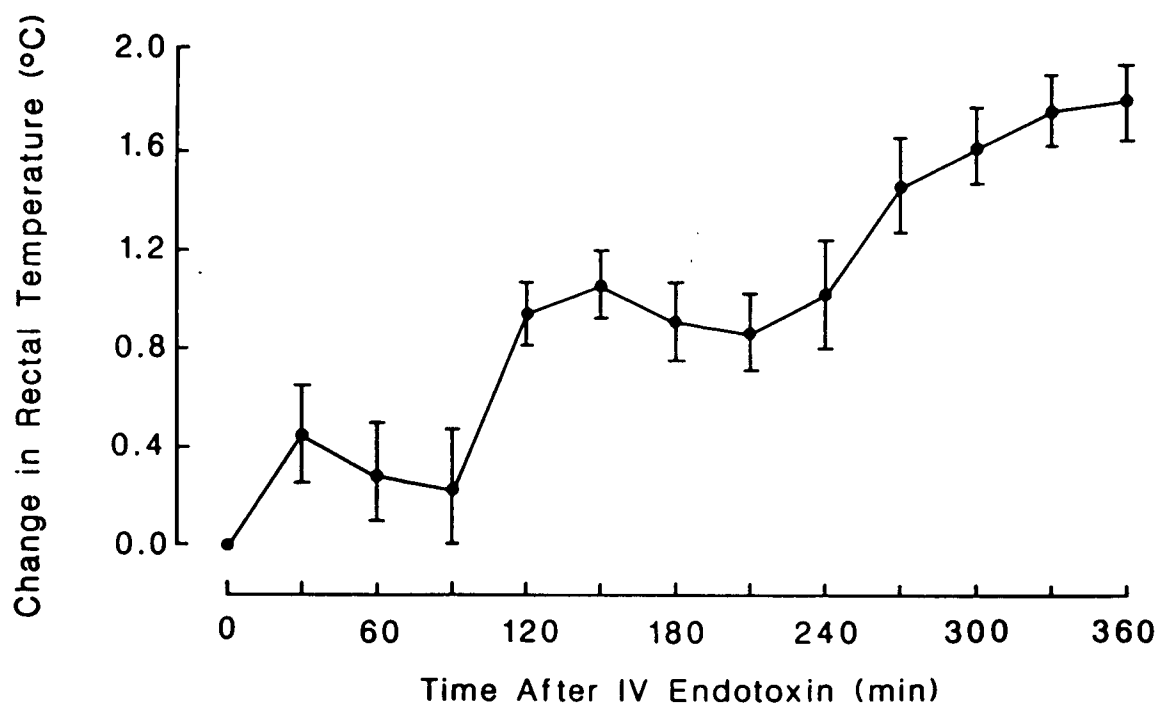
FIGURE 23

The CSF AVP release profile following iv endotoxin at time 0 (n=6). There were no statistical differences between febrile and non-febrile groups. Values are expressed as mean $\pm$ SEM.
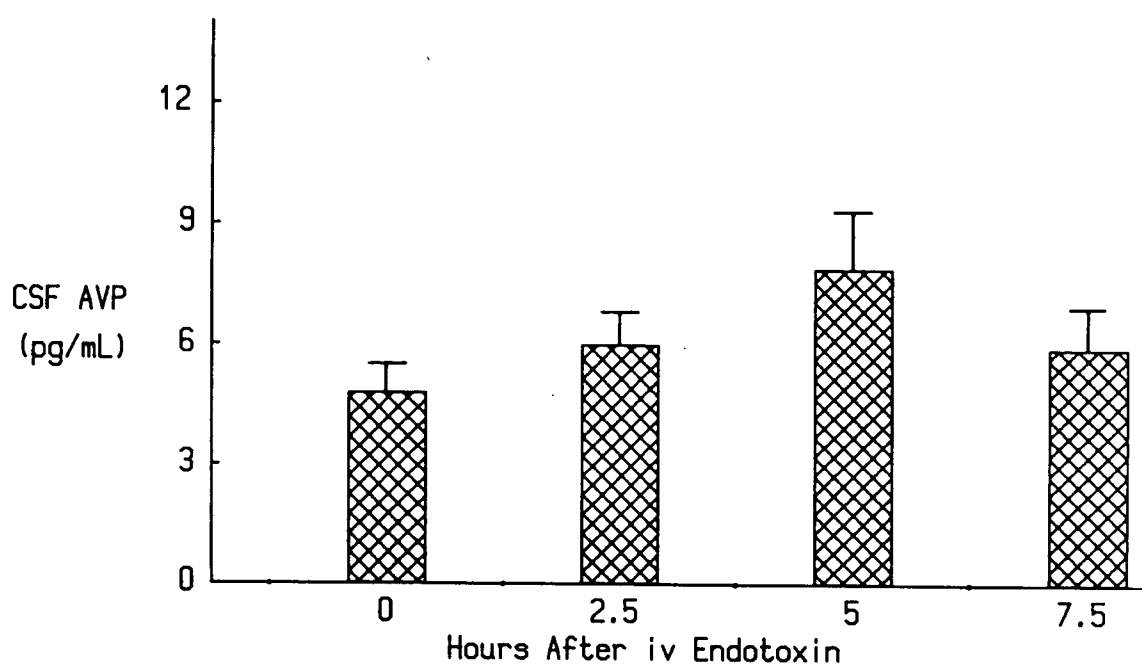
IV. DISCUSSION

A. Experimental Series 1. AVP Antagonist

These experiments demonstrate the marked antipyretic effect of AVP when administered icv in the rat. It has been observed previously that AVP, administered prior to fever inducement, can prevent the subsequent fever if perfused into a discrete brain site (Kasting et al, 1979c; Cooper et al, 1979; Naylor et al, 1985; Ruwe et al, 1985; Naylor et al, 1986) or if injected icv in the rat (Kandasamy and Williams, 1983; Kovacs and DeWied, 1983). The present data are the first to indicate that icv AVP can also reverse fever once it has risen above $1.0^{\circ}C$ and can do so at a dose that has no effect on normal body temperature. Curiously, icv administration of AVP in other animals is not antipyretic (Bernardini et al, 1983; Lee et al, 1985; Naylor et al, 1985). This may reflect the increased distance for diffusion of the peptide to the active site in the brains of larger animals rather than a difference among species to the effects of AVP.

Thus, AVP can both prevent a fever as well as reverse an established fever making it possible that the peptide could be acting via the neural elements responsible for the body temperature set-point. This is based on the knowledge that the effector mechanisms utilized to produce fever are different from those used to maintain a fever (Splawinski et al, 1977; Szekely et al, 1979; Stitt et al, 1985).From this evidence, it would seem that AVP is not altering one effector system specifically and must, therefore, be influencing the body temperature set-point.

The antipyresis observed after icv AVP appears to be of central origin since the dose used has no effect on either heart rate or blood pressure (DeWied et al, 1984; Zerbe and Feuerstein, 1985). This is in contrast to larger hypothermic doses used in the non-

febrile rat (Kasting et al, 1980b; Meisenberg and Simmons, 1984) where peripheral cardiovascular events may be mediating the observed temperature effects (Shido et al, 1984). Furthermore, central blockade of AVP receptors using a specific AVP, V_1-antagonist, delivered icv, could not abolish the hypothermic effect of AVP delivered via the same route (Meisenberg and Simmons, 1984).

Icv administration of the same V_1-antagonist (M-AVP) in febrile rats, was shown to abolish the AVP-induced antipyresis. This antagonism was effective if M-AVP was given 2 min or 24 h prior to AVP treatment. This strongly suggests a specific, centrally located, receptor mediated mechanism of action. The long duration of action observed in this study has also been previously been reported for M-AVP (Kruszyinski et al, 1980).

Injection of M-AVP alone in febrile or non-febrile rats had no effect on Tb. This suggests that the observed antagonism of AVP-induced antipyresis was not merely due to a thermogenic action of M-AVP. In addition, centrally administered M-AVP at equivalent doses has no cardiovascular effects which might contribute to the observed antagonism of AVP-induced antipyresis (Rockhold et al, 1984; King et al, 1985; Morris et al, 1986).

These experiments also employed the antipyretic drug indomethacin, in conjunction with M-AVP, to assess the possible interaction between the endogenous AVP system and drug-induced antipyresis. It was hypothesized that indomethacin could act either (1) by stimulating endogenous AVP release or (2) by interactions with the post-synaptic neurons of the AVP system. Two experiments were conducted to test these possibilities.

If indomethacin was acting by increasing endogenous AVP release then the AVP antagonist would be expected to prevent antipyresis induced by indomethacin. While this did not occur, the hypothesis cannot yet be discarded since it has been shown that to

effectively manipulate the endogenous AVP system, direct application of M-AVP into the discrete brain site is required (Cooper et al, 1987). Other investigators employing the same antagonist have also shown that icv administration of M-AVP is ineffectual at modifying endogenous AVP activity (Rockhold et al, 1984; King et al, 1985).

The other possibility for indomethacin-induced antipyresis is that the drug could interact with the post-synaptic neurons of the AVP system. Since endogenous AVP activity is negatively correlated with fever height (Cooper et al, 1979), the AVP activity should be low during an untreated fever. Thus, if exogenously applied AVP is given during drug-induced antipyresis this might be expected to decrease body temperature further. Moreover, application of the antagonist under these conditions should have minimal effects since endogenous AVP activity is low. While no increases in antipyresis were observed during a combined AVP-indomethacin treatment, the antagonist was ineffective at modulating drug-induced antipyresis. This would be expected if endogenous AVP activity were low. Again, direct application of M-AVP into the VSA will allow a more definitive analysis of the possible interactions between drug-induced antipyresis and the endogenous AVP system. The lack of effect of M-AVP on fever height further indicates the need for direct tissue injection to manipulate the endogenous AVP system, since Cooper et al (1987) were able to enhance fever by injecting M-AVP directly into the VSA.

B. Experimental Series 2. AVP at Different Ambient Temperatures

At an ambient temperature of $25^{\circ}C$ icv AVP caused antipyresis which was dose dependent. The variability in AVP-sensitivity between different groups of animals made it necessary to continue studies using a lower, but still markedly antipyretic dose, of the peptide.

Having established a working dose, experiments were carried out to determine the effects of different ambient temperatures on the antipyretic effects of centrally administered AVP. An antipyretic is defined as a substance which can reduce the body temperature of a febrile subject but has no effect on normal non-febrile temperature unless the dose is excessive (Barbour, 1921). These data show that icv AVP was an effective antipyretic agent at room temperature (25°C) according to the preceding definition. This is in agreement with previous reports (Kandasamy and Williams, 1983; Kovacs and DeWied, 1983; Kasting and Wilkinson, 1986). Since AVP has no effect on the normal body temperature, it is possible that at 25°C the peptide could be (1) decreasing febrile set-point, (2) inhibiting febrile heat production, or (3) inhibiting febrile cutaneous vasoconstriction. Since it was observed in these experiments that fevers produced predominantly by increases in heat production (at 4°C) were affected to a greater degree by AVP than fevers produced by decreases in heat loss (at 32°C), this suggests that AVP alters specific thermoregulatory effectors. If AVP were acting strictly on set-point, one would expect an equal decrease at all three ambient temperatures or an even greater decrease at 32°C than at 25°C or 4°C since fevers were greatest at 32°C (2.2°C) compared to 25°C (1.4°C) or at 4°C (1.1°C). This did not occur.

It is nonetheless true that at all three ambient temperatures, AVP consistently decreased the temperature of febrile more so than non-febrile animals. This, along with the observations that AVP can both prevent and reverse an established fever, suggests that AVP may indeed have some effect on febrile set-point.

It has been shown that AVP given into the lateral septum in a non-febrile rat model depressed metabolic heat production whereas vasculature tone of cutaneous vessels was unaffected (Banet and Wieland, 1985). This is hard to interpret with regards to the present study, because hypothermia was elicited by AVP in non-febrile rats which is in

contrast to these results. Also, injections were in sites not known to be sensitive to the antipyretic action of AVP. This may reflect use of larger hypothermic dose in contrast to a smaller antipyretic dose used in this study.

If AVP is antipyretic by virtue of a mechanism of action similar to that of a non-steroidal antipyretic drug then the qualitative responses to icv AVP and sc indomethacin should be similar at each of the three ambient temperatures. The responses to AVP and indomethacin were, in fact, very similar at ambient temperatures of $25^{\circ}C$ and $32^{\circ}C$ whereas at $4^{\circ}C$ both substances resulted in a decrease in brain temperature (Tb) of non-febrile rats although this decrease was not statistically significant after AVP. AVP was a more effective antipyretic at $4^{\circ}C$ than indomethacin, in that, it remained more effective at reducing the temperature of the febrile than non-febrile animals. As noted in the definition provided above, antipyretics can often decrease body temperature of non-febrile animals with higher doses. It may be that the results obtained at $4^{\circ}C$ are dose-related, that is, the rat is more sensitive to the effects of antipyretics in the cold, and that by using a lower dose of both substances, we might find that the antipyretic effects can be separated from the hypothermic effects. Thus, indomethacin may also have effects on specific thermoregulatory effector mechanisms. It has been previously observed that both indomethacin (Cranston et al, 1975) and salicylate (Satinoff, 1972; Francesconi and Mager, 1975) caused hypothermia in cold exposed animals. It was suggested that this hypothermia might be due to a prostaglandin mediated increase in heat production (Satinoff, 1972) or to an increased heat loss brought about by the salicylate (Francesconi and Mager, 1975). Evidence contrary to the prostaglandin hypothesis has been reported (Cranston et al, 1975).

Although observations in the cold indicate there may be differences in antipyretic mechanisms, these data do not provide any convincing evidence that AVP acts in a fundamentally different way from the non-steroidal antipyretic drug, indomethacin. Indeed, the infusion of another non-steroidal antipyretic, sodium salicylate, within the VSA, the neural area sensitive to the antipyretic effects of AVP, can suppress prostaglandin-induced fever (Alexander et al, 1986). Furthermore, the same prostaglandin-mediated fever can be suppressed if AVP is perfused within the VSA (Ruwe et al, 1985). From this, it can be concluded that both AVP and antipyretic drugs may be acting on the same neural pathways to have their antipyretic effects.

These data suggest that AVP lowers febrile temperatures in a manner similar to a known antipyretic drug, indomethacin. The evidence presented from these experiments also indicate that AVP may have effects on both specific effector mechanisms and on febrile set-point. Confirmation of these conclusions depends on measurement of specific effector mechanisms under similar conditions.

C. Experimental Series 3. Thermoregulatory Effector Mechanisms

From the above discussion it is clear that icv AVP in the rat produces varying degrees of antipyresis depending on the ambient temperature. AVP decreased Tb of febrile animals more so than the Tb of non-febrile animals at all ambient temperatures tested. Moreover, the actions of this neuropeptide were qualitatively similar to a known antipyretic drug, indomethacin. From these observations and the experiments which show AVP can both prevent (Kandasamy and Williams, 1983; Kovacs and DeWied, 1983) and reverse febrile changes in Tb, it is possible that AVP may be antipyretic by altering the febrile set-point. However, the observations that antipyresis was greatest in the cold (4°C) and least in the warm (32°C), suggested there may be a component of this effect which

depended upon a specific, yet undetermined, effector mechanism. These experiments sought to test this hypothesis by measuring effectors involved in both heat loss and heat production during AVP-induced antipyresis. The evidence presented here strongly supports the idea that the antipyretic effects of AVP are mediated by an effect on febrile set-point.

At $25^{\circ}C$ the antipyretic effect of icv AVP, observed in febrile rats, was accompanied by tail skin vasodilation, an indication of heat loss effector stimulation. This is the first evidence of centrally administered vasopressin producing a vasodilatory effect. At higher doses, both peripherally (Shido et al, 1984) and centrally (Zerbe and Feuerstein, 1985), this peptide typically causes vasoconstriction. No changes in M, an index of heat production, were observed in febrile rats receiving AVP at this ambient temperature. Previous reports using higher doses of vasopressin administered intravenously (Shido et al, 1984) or into the lateral septum (Banet and Wieland, 1985) of non-febrile rats have shown the peptide to suppress heat production while leaving peripheral vasomotor tone unaffected. These results demonstrate fundamental differences in both sensitivity and the type of response to AVP in febrile versus non-febrile rats under these conditions.

At $4^{\circ}C$, Tb of febrile rats decreased more so than in febrile rats at $25^{\circ}C$ and $32^{\circ}C$ despite cutaneous vasoconstriction throughout the period of observation. It can be concluded from this that the inhibition of M in febrile rats following AVP was responsible for the observed antipyretic effect. It is interesting to note that the level to which M decreased after AVP in the cold was similar in magnitude to the M of rats at $25^{\circ}C$ which was unaffected by AVP. This may indicate that M can only be inhibited when it is above a certain threshold. The paradoxical vasoconstriction at $4^{\circ}C$ may be a reflex response to the rapidly falling Tb.

The small but significant antipyresis elicited by AVP at 32°C could not be shown to be due to changes in either Tsk, M or EHL. At this ambient temperature heat production is at a minimum and heat loss is at a maximum. This is evident from the low values for M and the high values for Tsk at 32°C. Tsk at 32°C was very close to the mean Tb and therefore could not be expected to increase further. Under these conditions it is difficult for the animal to eliminate significant amounts of heat. Our results confirm the difficulty of losing heat since the dose of AVP could clearly elicit antipyresis at lower ambient temperatures. It is possible that the subtle changes in heat loss and/or heat production necessary to produce this small degree of antipyresis are below the limits of resolution of our experimental procedures. The other possibility is that each rat uses a slightly different heat loss strategy and the calculation of means prevents the changes from being distinguishable.

The results of this study provide evidence that in the febrile rat, centrally injected AVP is initiating changes that are aimed at reducing body temperature. The thermoregulatory effectors that are utilized to attain this goal differ depending on the ambient temperature. In other words, the heat loss strategy differs at each ambient temperature and AVP does not simply activate the same effector under all conditions. This supports the hypothesis that AVP is antipyretic due to its action on febrile set-point rather than on a specific effector system. The neural/neurochemical basis for the thermoregulatory set-point has not been clearly established so the mechanism of action by which AVP affects set-point remains to be determined.

D. Experimental Series 4. AVP Levels in CSF

AVP is present in the CSF of many species including man (Amico et al, 1985; Sorensen et al, 1985), monkey (Reppert et al, 1983; Perlow et al, 1982), cat (Reppert et al,

1981; Reppert et al, 1982; Reppert et al, 1983), rabbit (Gunther et al, 1984) and the rat (Mens et al, 1982; Reppert et al, 1983; Schwartz et al, 1983). Except for humans, a circadian rhythm of AVP release has been observed in all species examined. While the presence of AVP in the CSF has undetermined physiological significance, it has been suggested that the CSF might provide a pathway for neuroendocrine integration (Rodriguez, 1976). This hypothesis is based on the periventricular anatomy where ependymal and neuronal formations are specialized for both secretory and transport processes. Specifically, CSF AVP has been suggested to play a putative role in learning and memory processes (Dogterom et al, 1977; Schwartz et al, 1983). The purpose of the present experiments was to determine if AVP, released into the CSF, was affected during fever and whether the endogenous peptide could be contributing to the antipyresis elicited by exogenous AVP.

AVP was detectable in the cisternal CSF of conscious and unrestrained rats. The release pattern for the neuropeptide exhibited a daily rhythm similar to that seen previously (Mens et al, 1982; Reppert et al, 1983; Schwartz et al, 1983). The AVP concentrations in this study agreed closely with Reppert et al (1983) and Schwartz et al (1983) but were much lower than those reported by Mens et al (1982). The reason for this discrepancy is unclear.

In rats treated with endotoxin iv, a fever developed which exhibited a typical biphasic pattern. Although this febrile response differs from the monophasic pattern of endotoxin icv, a previous study in the sheep reported an enhanced CSF AVP release to endotoxin administered iv (Kasting et al, 1983). The present study sought to examine this response in the rat using similar procedures.

The present results do not agree with the findings of Kasting et al (1983). In CSF from the febriie rat, there were no statistical differences in AVP concentrations compared to non-febrile controls. However, the sheep study did not follow AVP release in the non-febrile animal and it is therefore possible that their results are a reflection of the normal daily release of AVP into the CSF of the sheep. No study, to my knowledge, has examined the circadian release of CSF AVP in the sheep. Another interpretation of this apparent discrepancy is that AVP measured in CSF sampled from the lateral ventricle, as in the sheep study, could differ from AVP levels measured in cisternal CSF. Indeed, studies have shown that differences do exist in peptide concentrations between CSF compartments, specifically the third ventricle and the cisterna magna (Szczepanska-Sadowska et al, 1983; Szczepanska-Sadowska et al, 1984).

AVP exhibits no rhythmic release into the plasma (Schwartz et al, 1983) but is released into the plasma in response to endotoxin (Kasting et al, 1985; Kasting, 1986b). There is, however, an effective blood-CSF barrier which precludes significant amounts of the peptide from entering the CSF via the plasma (Ang and Jenkins, 1982; Mens et al, 1983). The most likely source for CSF AVP and the probable generator of the rhythmic release is the suprachiasmatic nucleus (SCN) (Schwartz and Reppert, 1985).

The results of these experiments indicate that during fever CSF AVP is not influencing the thermoregulatory process since CSF AVP release was no different in febrile versus non-febrile rats. This is substantiated by the lack of effect of icv administration of M-AVP in either febrile or non-febrile rats. Thus, despite V_1-receptor blockade, temperature was unaffected. Together these results lead to the conclusion that AVP circulating within the CSF has no functional role in temperature regulation.

In summary, the results of this thesis have demonstrated that icv AVP is antipyretic and is similar in this effect to a known antipyretic drug, indomethacin. Furthermore, it is concluded that AVP induces antipyresis by its action on febrile set-point rather than on a specific effector system. This action of AVP is mediated by a V_1-like receptor mechanism which is not stimulated by endogenous CSF AVP. The neural/neurochemical basis for the thermoregulatory set-point has not been clearly established so the mechanism by which AVP affects set-point remains to be determined. These data contribute, however, to the growing body of evidence that AVP is acting centrally as a neurotransmitter or neuromodulator to affect body temperature during the febrile process.

V. Summary Statements and Conclusions

1) A rat model was developed to investigate the antipyretic effects of intracerebroventricularly (icv) administered arginine vasopressin (AVP). Icv AVP caused antipyresis in rats with fever induced by icv endotoxin. Previously, AVP pretreatment had been shown to prevent endotoxin fever. Thus, this is the first evidence to demonstrate that AVP can reverse an established fever as well as prevent onset of the fever . This suggests that the neuropeptide may be acting on febrile body temperature set-point mechanisms.

2) A V_1-AVP receptor antagonist, M-AVP, administered icv prevented AVP-induced antipyresis. The antipyretic effects of AVP are therefore receptor mediated and deemed likely to be of physiological importance.

3) The V_1-antagonist administered icv did not alter fever height or indomethacin-induced antipyresis. Subsequent experiments in another laboratory have shown that the AVP antagonist must be microinjected directly into the tissue of the brain to affect fever height. This evidence, therefore, points out that neuroactive substances given into the ventricular system do not necessarily have access to different areas of the brain.

4) AVP decreased febrile more so than non-febrile body temperatures at ambient temperatures of $4^{\circ}C$, $25^{\circ}C$ and $32^{\circ}C$. This suggests that AVP has an action on febrile set-point but may also affect a specific, but undetermined, effector.

5) The antipyretic drug, indomethacin, administered subcutaneously, induced qualitatively similar effects to AVP, suggesting a similar mechanism of action.

6) AVP was administered icv in febrile rats at three ambient temperatures during measurements of brain temperature, tail skin temperature and oxygen consumption. At $25^{o}C$, AVP-induced antipyresis was accompanied by vasodilation while oxygen consumption remained unaffected. At $4^{o}C$, simultaneous vasoconstriction and heat production inhibition was observed during AVP-induced antipyresis. At $32^{o}C$, no changes were observed in heat production, tail skin temperature or evaporative heat loss following AVP treatment in febrile animals. This evidence demonstrates that rats use different effectors, depending on the ambient temperature, to mediate antipyresis. This strongly suggests that AVP alters febrile set-point.

7) CSF AVP was measurable in unrestrained conscious rats but was not altered during fever. It therefore seems unlikely that ventricular AVP has a role in fever modulation.

8) The data presented in this thesis suggest that icv AVP induces antipyresis by an action on febrile set-point. This effect is mediated by a V_1-like receptor mechanism which is not normally mediated by endogenous CSF AVP. Since the neurophysiology and neuroanatomy of the set-point remain to be clearly established, the mechanism by which AVP affects the set-point is unknown. These data, however, contribute to the growing body of evidence suggesting that AVP is acting centrally as a neurotransmitter or neuromodulator to affect body temperature during the febrile process.

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