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Central Nervous System Effects of Sodium Salicylate on Prostaglandin-Induced Hyperthermia

by

Susan J. Alexander

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled, "Central Nervous System Effects of Sodium Salicylate on Prostaglandin-Induced Hyperthermia", submitted by Susan J. Alexander in partial fulfillment of the requirements for the degree of Master of Science.

Dr. W.L. Veale, Supervisor

Department of Medical Physiology

Dr. K.E. Cooper

Department of Medical Physiology

Dr. H. Koopmans

Department of Medical Physiology

Dr. B.H. Bland

Department of Psychology

November, 1986

ABSTRACT

The antipyretic effect of sodium salicylate infused within the ventral septal area (VSA) of the rat brain was investigated. The VSA had been previously shown to be a sensitive area of tissue where arginine vasopressin (AVP) and prostaglandins of the E series (PGE) could reduce and produce fever, respectively. Furthermore, PGE fever (icv) has been suppressed by push-pull-perfusion of AVP within the VSA. It was not known what effect sodium salicylate within the VSA would have on fever.

Infusion of artificial cerebrospinal fluid (aCSF) or sodium salicylate within the VSA was started 1.0 h prior to and continued for 1.0 h following microinjection of 200.0 ng of PGE_1 (20.0 ng/µl). The effects on deep body temperature were monitored continuously. During infusion of aCSF, PGE evoked fevers of approximately 1.0 °C. The infusion of sodium salicylate however, significantly reduced PGE-induced fevers. The salicylate-induced blockade of PGE-induced hyperthermia was site specific in that infusions more dorsal to the VSA, near the anterior commissure did not result in suppressed PGE hyperthermia.

A dose effect test indicated that the reduction of PGE-induced hyperthermia by sodium salicylate within the VSA was a dose-related effect. Artificial CSF and 10.0 µg doses of salicylate did not reduce PGE fevers whereas 30.0, 50.0 and 100.0 µg doses diminished the PGE hyperthermia, as measured by the fever index. There have been some reports in the literature that sodium salicylate administered intraperitoneally induced marked hypothermia in rats. The possibility

that such an hypothermic effect was responsible for the salicylate-induced reduction of icv PGE fever in the present experiments was therefore investigated. At ambient temperatures of 10.0 °C the infusion of sodium salicylate within the VSA did not reduce rectal temperature to a significant degree. The results were taken as evidence that salicylate did not lower normal body temperature when infused within the VSA of the rat.

Sodium salicylate is thought to exert its antipyretic effect by inhibiting the synthesis and release of prostaglandins. If this were the sole mechanism of action of sodium salicylate then the present results should not have occurred. Therefore, it was postulated that salicylate was blocking exogenous PGE via another mechanism. One possibility was that sodium salicylate was enhancing the effects of the endogenous antipyretic peptide, AVP, which is present within the perfused area of the ventral septum. This notion was tested by infusing AVP antiserum or AVP (V1) receptor antagonist in combination with sodium salicylate into the VSA. The resulting PGE fevers occurred at levels which were not significantly different from control levels as opposed to enhanced fever (antiserum or antagonist alone) or suppressed fever (salicylate alone). These results were taken as supporting evidence for the notion that sodium salicylate infusions within the VSA enhanced endogenous AVP action therefore bringing about the blockade of PGE-induced hyperthermia. Several alternate mechanisms of action for sodium salicylate antipyresis are discussed.

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Dedication

To my mother for her love and unrelenting belief in me.

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I. Introduction

A. Regulation of Body Temperature

Thermoregulation is a homeostatic process in which afferent thermal information from peripheral and central sensors is integrated into efferent signals which allow core body temperature to be regulated. In the majority of mammalian species the balance of heat gain and heat loss is such that core temperature is held within very narrow and well guarded limits even during large changes in ambient temperature (Boulant, 1980).

The anterior hypothalamic-preoptic area (AHPOA) of the mammalian brain is essential for the regulation of body temperature. Indeed, "without the anterior hypothalamic-preoptic area's ability to transduce its own temperature into neural impulses there is no regulation of body temperature" (Hammel, 1968). The existence of thermosensitive neurons in the AHPOA was demonstrated early in the 1960s and investigation of these neurons has been extensive since then (Nakayama, 1985). Three general classes of thermosensitive neurons can be identified by electrophysiological means and these are warm- or cold sensitive and temperature insensitive neurons. The thermosensitive neurons of the AHPOA are influenced by local, peripheral, spinal and brainstem temperatures (Nakayama, 1985). There are extra-hypothalamic areas of the brain which have been indicated as thermosensitive including: the septum, the posterior hypothalamus, areas of the midbrain, pons and medulla (Boulant, 1980) and the spinal cord (Simon, 1974). Interactions among thermosensors, AHPOA neurons (and possibly other extrahypothalamic

neurons) and thermoeffectors occur so that core temperature is regulated in a strict and precise manner.

B. Body Temperature Set-Point Theory

The level at which mammalian body core temperature is held and maintained from birth to death is the set-point temperature. According to Bligh (1982) "despite our persisting ignorance of the nature of the processes involved, both in the creation of a set-point and in its adjustments..." the actual term is quite valid since it describes the performance of a system and not the means by which the performance is achieved (Bligh, 1982). The notion of a thermostat within the body was generated around 1871 by Liebermeister (cf Cooper, 1972a). In a classic treatise of 1876 Bernard (cf Lomax, 1979) recognized that higher animals maintained an equilibrated environment. Despite the early recognition and subsequent theorizing, little headway had been gained in understanding the mechanisms by which the core temperature was held at a set 37°C until 100 years later.

Based on experiments in which the ratio of sodium (Na^+) and calcium (Ca^{++}) was selectively altered in specific regions of the hypothalamus, Myers and Veale (1970) proposed that the set-point for body temperature was localized within the posterior hypothalamus (PH) and was determined and maintained by the inherent ratios of these two ions. An excess of Na^+ in the aqueous medium perfused within the PH resulted in shivering and increase in body temperature. In contrast, and excess of Ca^{++} in the perfusion medium resulted in a decrease in body temperature (Myers

and Veale, 1970). Subsequently it was shown that the PH was indeed the region of the diencephalon most sensitive to local alterations of ionic constituents and the ensuing changes in body temperature (Myers and Veale, 1971). Furthermore, the level at which a monkey's set-point was maintained could be varied by changing the proportions of Ca^{++} and Na^{+} in the CSF perfused through the PH (Myers, 1971). The responses that are elicited when the ratio of these two ions is changed in perfusion fluid and presumably extracellular fluid (ECF) appeared not to differ much among species (Veale and Cooper, 1973).

According to the set-point theory of Myers and Veale (1970), the readjustment of the set-point in fever would require that within the hypothalamus either a decrease in $[Ca^{++}]$ or an increase in $[Na^{+}]$ occurred. Indeed, such a change was shown in that bacterial pyrogen (BP) could act directly on the brain tissue to bring about a disturbance of the sodium/calcium ratio in ECF (Myers and Tytell, 1972). As temperature increases induced by icv BP were occurring the efflux of radiolabelled Ca^{++} from diencephalic tissue increased greatly whereas Na^{+} efflux fell. Importantly, the increase in Ca^{++} efflux and decrease in Na^{+} efflux could be reversed by the administration (ip) of the antipyretic acetaminophen (Myers, 1976). Clearly, the Na^{+} and Ca^{++} ratio within the brain seemed to be a likely mechanism to explain temperature set-point during normal and febrile conditions. Later it was shown that extreme changes in ambient or hypothalamic temperatures effected the efflux of Ca^{++} as did thermogenic agents such as BP, PGE and seratonin administered in the AH/POA (Myers, 1978). These experiments pointed to the ability of the Ca^{++} ion mechanism and Na^{+}

activity to operate whenever the neuronal input from peripheral areas signals a severe thermal challenge to the rostral hypothalamus (Myers, 1978).

The numerous experiments done by Myers through the 1970s were supported and extended by Jones et al., (1980). They examined several possible explanations for effects seen during the alteration of the ionic milieu in the PH including: that increased Ca^{++} increased transmitter release from synaptic vesicles; that increased Ca^{++} or decreased Na^{+} stabilized the neuronal membranes making the cells less excitable; or that the effect was specific to thermoregulatory pathway neurons in the PH. Perfusion of ouabain, A23187 (a calcium ionophore) or tetrodotoxin within the caudal hypothalamus rendered results which indicated that synaptic events were not responsible for the changes seen in temperature. Rather, responses were due to neural effects mediated through increased or decreased firing of specific neurons or interneurons (Jones et al., 1980).

It can be seen that although the specifics of the set-point mechanism are still being worked out, there are several assumptions which have been made about the nature of the set-point function (Myers, 1980).

1. The site of the set-point mechanism is anatomically specific and separate from neuronal structures responsible for regulatory functions
2. The set-point temperature can be lowered or elevated by physiological or pathological events

3. Temperature regulation at a new set-point proceeds unaffected
4. The set-point temperature in mammals is usually present at birth
5. The set-point has a universal nature and should be governed by a fundamental and intrinsic property of a select population of neurons which are anatomically proximal to temperature-sensitive neurons
6. The set-point temperature can be shifted without serious or permanent pathological consequences.

In conclusion, the evidence suggests clearly that the PH is the locus of the set-point mechanism. When a thermal challenge is present there appears to be transmission of impulses from the anterior to the posterior hypothalamus which results in a change in the activity of the ionic mechanism. The alteration in Ca^{++} kinetics of the PH would seem to lead to the well defined physiological responses which establish the new set-point temperature (Myers, 1982).

C. Fever

Temperature regulation predominates under numerous stressful states, one of the most common and well-known of which, is fever. Fever is defined as a pathological state in which body temperature is elevated to a new and higher level and then regulated at this new level (Veale et al., 1983). Thus, the febrile body is capable, even in response to environmental warmth and exercise, of regulating its temperature at the new higher level with considerable precision (McPherson, 1959; Stitt, 1979). Fever is referred to in this thesis as a pathophysiological elevation in set-point as opposed to excessive heat production over heat

loss or to defective heat loss (Done, 1983). Fevers of the high set-point nature are caused by infectious agents which lead to the production of endogenous pyrogens which then are thought to act upon the anterior hypothalamic preoptic area themselves or to induce formation and or release of pyrogenic substances which then act at the AHPOA.

i. Brief History of Fever Research

The pursuit of knowledge concerning the causes of fever and the ability to thermoregulate more or less proceed along similar lines from the earliest recorded accounts of fever through to the most recent characterizations of peptide effects on febrile and normal temperatures. The study of the history of fever contributes then to the understanding of the development of thermoregulatory theories.

Historically, fever has been described or referred to in association with disease for thousands of years. Symbols of fever were used in Akkadian cuneiform inscriptions of the 6th century B.C. and Celsus, a learned man of the early Roman Empire, depicted the four features of inflammation including, redness, swelling, pain and heat (cf Atkins and Bodel, 1979). Hippocratic writings are reputed to have contained very precise descriptions of "fever diseases" which were thought to be the result of an excess of bile (cf Signal, 1978). Later, Galenic theory attributed fevers to an overabundance of one of the humors, that is, blood, choler, melancholy or phlegm (cf Sigal, 1978). Galen's humoral theory persisted for 1500 years until the 17th Century.

Galen's theory of fever was uprooted when William Harvey theorized the existence of a closed circulatory system. This idea greatly affected the understanding and treatment of fevers in the 17th century. In an attempt to explain the disease of fever within the context of the circulatory theory, doctors theorized that fever was caused through obstruction of the body channels by solid particles (cf Signal, 1978). Practices of blood-letting to purge the body of its excess humors were soon frowned upon and some physicians preferred to let nature take its course because the thinking of the time was "most curable diseases are cured by nature and time". Another group of physicians and theorists, whose most well known member was Dr. Thomas Willis, put forth the theory of fever based on a fermentation in the blood: "I call an over-great or preternatural fermentation, when blood (like a pot boiling over the fire) boils above measure, and being rarefied with a frothy turgescence, swells the vessels, raises a quick pulse, and, like a sulphureous liquor taking fire, diffuses on all sides a burning heat" (cf Sigal, 1978).

Thus, 17th century thinkers claimed that fermentation of blood within the heart was the source of fever and fever itself was the disease. If the temperature of the fermentation fever itself was not enough to dissolve the alien matter in the blood then an evacuation technique such as vomiting or blood-letting was employed. The 17th century had yet a third group of physicians who preferred to observe each case and treat them accordingly with potions and concoctions (Sigal, 1978). The advent of treating fever with various potions led to great advances in pharmacology and the discovery of the antipyretic nature of the bark of the cinchona tree (quinine). Yet another line of

thinking in the 17th century with regard to fever was that of the mechanists such as Descartes. They believed that there was a central fire in the heart which then flowed to the brain through hollow neuronal tubes and powered the muscles of the body (cf Lomax, 1979). Toward the end of the 17th century however this line of thinking faded since Giovanni Borelli used a primitive air thermometer to measure the heat of the heart and other organs of a stag and found no temperature differences (cf Lomax, 1979).

The 18th century began with the theory that internal body heat was created by friction of red corpuscles against vessel walls but the theory was considered to be too mechanistic and chemical theories which were more likely to be proven were adopted. Late in the century a number of important physiological observations were made. Lavoisier, around 1780, combined a number of observations of his time to theorize that absorption of oxygen through the lungs (respiration) was analogous to combustion and that it provided the source of animal heat (cf Hardy, 1972). Lavoisier observed that a guinea pig placed in a cold chamber for a lengthy period of time did not emerge cold, as inanimate objects did, but rather at the same temperature it went in at (cf Fulton and Wilson, 1966). Shortly after this, Charles Blagden carried out experiments on humans which showed that the body's temperature could rise due to heat exposure without causing sickness or death, which was the current thinking at the time. He noted responses to extreme heat such as sweating and increased respiration (cf Fulton and Wilson, 1966). Similar experiments were performed on animals during the same year by Dobson, with similar results. Two physicians of the late 1700s, John

Hunter and James Currie, began the practise of measuring body temperature by means of a mercury-in-glass thermometer placed under the tongue (cf Holdcroft, 1980; cf Cooper, 1971). Febrile temperatures had not been measured until this clinical use of the thermometer began (cf Lomax, 1979). Such use must have led Currie to originate the idea that temperature was a regulated function.

Early in the 19th century Brodie postulated that the nervous system was involved in heat production although he could not imagine the nature of the connection. However most physiologists retained Lavoisier's theory that heat was produced by the combustive effects of respiration rather than accept the concept of a vital (nervous) element as the cause of body heat. Debate as to whether heat production was physicochemical or vital continued throughout the 1800s with physiologists such as Carpenter insisting that heat production was entirely a physical character and that the nervous systems had other important operations, not physical, to do (Carpenter, 1842 from Lomax, 1979). Magendie meanwhile acknowledged vital action but suggested that nothing of the internal molecular motions could be determined and therefore attention should be fixed upon the resulting effects of vital actions (Magendie, 1831 from Lomax, 1979). In 1871 Wunderlich presented his monumental work which was the summation of his body temperature observations of nearly 25,000 patients. It was a detailed study of fever during different diseases and again stressed the clinical importance of body temperature (cf Fulton and Wilson, 1966).

At about the same time (1871) Karl von Liebermeister hypothesized a mechanism of temperature regulation which suggested that temperature is reset at a new, higher value during fever (cf Fulton and Wilson, 1966). This notion was more clearly described by Lefevre in 1911 and has since been verified many times. Resetting of the thermostat is an established idea in fever development. Following this long line of physiologists and culminating the data and observations to that point in time, Claude Bernard, put forth a unifying concept which thereafter provided the basis for past observations and future physiological discoveries (cf Lomax, 1979). Bernard was the first to describe animals as having two environments: a "milieu exterieur" in which the organism is situated, and a "milieu interieur" in which the tissue elements live. Bernard also recognized that animal heat was an essential vital condition of the "milieu interieur" and that heat production was exerted through nerves (cf Lomax, 1979). Furthermore, his discussion of the "milieu interieur" also pointed out that higher animals maintain an equilibrated environment as a "result of compensation established as continually and as exactly as if by a very sensitive balance" (Bernard, 1876 from Fulton and Wilson, 1966). We may consider this the first discussion regarding the maintenance of body temperature through the balance of heat production and heat loss. It was later, in 1931, that the actual term "homeostasis" was coined by Cannon.

ii. Central Nervous System Site of Fever Production

While Bernard was formulating his landmark commentary, scientists had kept up the search for a controlling nervous element for temperature and fever. Chossat continued Brodie's lead by sectioning at various

levels of the spinal cord and observing a reaction comparable to the onset of fever (cf Lomax, 1979). Brodie then published again in 1837 confirming Chossat's observation in animals as well as adding his own similar findings from clinical studies of patients with spinal cord injuries. In 1866 Tscheschichin had found that cutting between the medulla oblongata and pons of rabbits caused temperature to rise. He concluded therefore that the heat producing capacity of the medulla must be moderated or controlled by a center higher in the brain. Late 19th century physiologists also began to look for the site of action of pyrogenic substances suspecting that both temperature regulation and fever development occurred in the same part of the central nervous system (CNS).

In 1904 Richet contributed two important thoughts to fever research: the first was that there was a distinction between microorganisms themselves being pyrogenic and an indirect effect of the microorganism on tissues which then produced febrile substances; the second postulate was that fever arose from a disturbance of one or more regulatory centers in the CNS. Exploration of such areas was fostered by the introduction of a new method of studying fever development. Using the heat puncture method many brain sites were subsequently tested for fever responses (cf Lomax, 1979). As well, brain areas were heated and/or cooled, their effect on temperature noted and compared to fever. Barbour, in 1912, observed that the hypothalamus was the only area to respond to cooling by increasing body temperature (cf Grundmann, 1969). Thus, with careful observation and the descriptions made by clinicians of the time, with exploratory and electrical stimulation experiments

made by physiologists, the possibilities for a temperature-regulating center were narrowed to the hypothalamus and the corpus striatum.

The hypothalamus was soon recognized as the area of the brain which was altered during fever. In 1921 another classic manuscript authored by Henry Barbour postulated that pyrogenic substances act within the brain to cause fever, that is, "normal temperature becomes apparently interpreted as cold by the temperature centers while one of 40°C perhaps feels neutral" (Barbour, 1921). Direct evidence that fever was brought about by central actions was later provided by King and Wood (1958) when they found that endogenous pyrogen caused a more rapid and pronounced fever when given via the carotid artery rather than intravenously. Meanwhile physiological, anatomical and clinical evidence accumulated which pointed to the hypothalamus as the major controller of normal and febrile body temperatures.

iii. Characteristics of the Febrile Response

In 1954, Fox and Macpherson described the characteristic ability of the body to continue thermoregulating even during a fever. That is, the elevation of body temperature during fever was actively defended in a normal manner. Another characteristic of fever was that it could be generated at any ambient temperature and that for a given dose of pyrogen the increase in body temperature was largely independent of ambient temperature (Grant, 1949; Cooper, 1972b). A further characterization of fever was a rapid upward shift in the subject's thermal perferendum which was then relieved by both autonomic and behavioral mechanisms (Cooper et al., 1964; Atkins and Bodel, 1972;

Crawshaw and Stitt, 1975). When the preferred temperature is reached it is actively defended at that level without impairment to the normal functioning of thermosensor or thermoeffector elements (Cooper et al., 1964; McPherson, 1959; Stitt, 1979).

D. Endogenous Pyrogen

The discovery of endogenous pyrogen (EP) was made by Beeson in 1948 when he found that upon exposure to exogenous pyrogenic substances the granulocytic blood cells of the body produced their own mediatory substance which was pyrogenic (Beeson, 1948). After him, other researchers made this observation using various cells of the reticulo-endothelial system (RES) to produce the endogenous substance, which was distinctly different from the exogenous pyrogens (Bennett and Beeson, 1953). It is now known that EP is a heat-labile protein of approximately 15000 daltons (Dinarello et al., 1977). EP is not preformed in RES cells (Bodel, 1970) rather, production of EP requires that the cells be activated by agents such as endotoxin or bacteria after which the actual production of EP begins (Gander, 1982). EP has been shown to circulate in various laboratory animals and humans during a variety of experimental fevers although its circulation in humans experiencing a natural fever during a disease state has not yet been detected (Dinarello and Wolff, 1982; Dinarello, 1984). EP is made by many vertebrate species and EP of one species will produce fever in another species (Dinarello et al., 1977). When given intravenously (iv) the fever produced is brisk but EP administered through the carotid artery results in a more rapid and pronounced fever than when injected

iv. This was the first indication that EP acts directly upon the CNS (King and Wood, 1958).

Experiments in which EP was applied directly to brain sites established that the anterior hypothalamic/preoptic area (AHPOA) was very sensitive to EP, causing fever in 7-8 minutes at a dose 1/100th of that required for a comparable fever with an iv injection. As well, other sites such as the posterior hypothalamus, mid-brain, cerebellum or cerebral cortex were not sensitive to the fever-inducing effects of EP (Cooper, 1965; Villablanca and Myers, 1965; Cooper et al., 1967; Jackson, 1967). Furthermore, bacterial pyrogen applied directly to the AHPOA resulted in a fever with an onset latency of 25 minutes (Cooper et al., 1967) to 5-20 minutes (Villabalnca and Myers, 1965) which is considerably longer than that of EP. Thus the particular sensitivity of the AHPOA to pyrogens has been well established (Jackson, 1967; Veale and Cooper, 1975). It is important to note that extrahypothalamic sites sensitive to pyrogens have been observed (Rosendorff and Mooney, 1971; Eisenman, 1969) however it has been suggested that these alternate sites are secondary to the AHPOA in the pathogenesis of fever (Dinarello and Wolff, 1982). That is, when the AHPOA is lesioned, the ability to regulate temperature is impaired although fever development due to iv EP still occurs (Cooper and Veale, 1974; Lipton and Trzcinka, 1976).

Currently then, the theory of fever is that activation of both fixed and circulating cells of the RES by exogenous pyrogens results in the production of EP. The EP is released into the bloodstream where it may cross the blood-brain barrier to effect, directly or indirectly, the

AHPOA (and possibly other areas). There is recent evidence which suggest that EP may cause a nonspecific increase in levels of intracellular calcium which then activates membrane phospholipases and leads to the production of arachidonic acid, the precursor substance for prostaglandins which may then act as a second signal for EP-induced cell activation (Dinarello, 1984). Further evidence which suggests that EP has an indirect effect has been found. For example:

1. Radiolabelled leukocytic pyrogen given iv in rabbits does not appear in the AHPOA or a lateral cerebral ventricle (LCV) at 15 or 60 minute sample times (Dinarello et al., 1978)
2. Prostaglandins of the E series are present in the CSF during pyrogen induced fevers (Feldberg et al., 1973; Bernheim et al., 1980; Coceani et al., 1983)
3. The delay of onset following EP injection into the brain is suggestive of another mediatory substance such as PGE which produces fever very rapidly when injected into the AHPOA in extremely small amounts (Cooper and Veale, 1974).

These observations suggest that EP may have its effect on the CNS via indirect mechanisms. Alternatively, new evidence has shown that EP or Interleukin-1 (IL1), to which it is now referred, is synthesized within the brain of mice when an intraperitoneal (ip) injection of bacterial pyrogen is given (Fontana et al., 1984). The presence of endogenous Il1 in the brain is compelling evidence for a direct effect of this pyrogen on central tissues. It may be that fever is the result of both direct and indirect actions being exerted by EP at various times during the course of fever (Mitchell et al., 1986).

The effect of EP (or substances released locally by EP) on the thermoregulatory neurons has been investigated thoroughly and it has been established that warm-sensitive neurons which respond to heating by increasing their rate of fire, respond to EP by decreasing their firing rate. Alternatively, cold-sensitive neurons, which respond to heating by decreasing their rate of fire, increase their firing rate in response to EP application (Eisenman, 1969; Schoener and Wang, 1975a; Hori et al., 1984). These changes in firing rate are consistant with the notion of decreased heat loss and/or increased heat production respectively. Presumably heat gain continues until these thermosensitive units return to control levels.

The fate of EP once it has been introduced into the brain seems to be excretion through the ependyma into the cerebrospinal fluid (CSF) of the ventricular system (Veale and Cooper, 1974b). Furthermore, current investigations have suggested that EP, like a classical hormone, can regulate its own production through a self-induced inhibitor, PGE_2 (Kunkel et al., 1986) suggesting that the brain has a mechanism by which levels of intra- and extracellular IL1 can be controlled. As well, there is evidence for the existence of an immunoregulatory feedback circuit in which IL1 acts as an afferent and glucocorticoids (which inhibit prostaglandins) act as an efferent hormonal signal (Besedovsky et al., 1986). It would seem that there is much about the effects of EP on central nervous tissue to be discerned. With regard to fever there are investigators who advocate that the fundamental biologic function of EP may be related to its ability to induce phospholipases, which

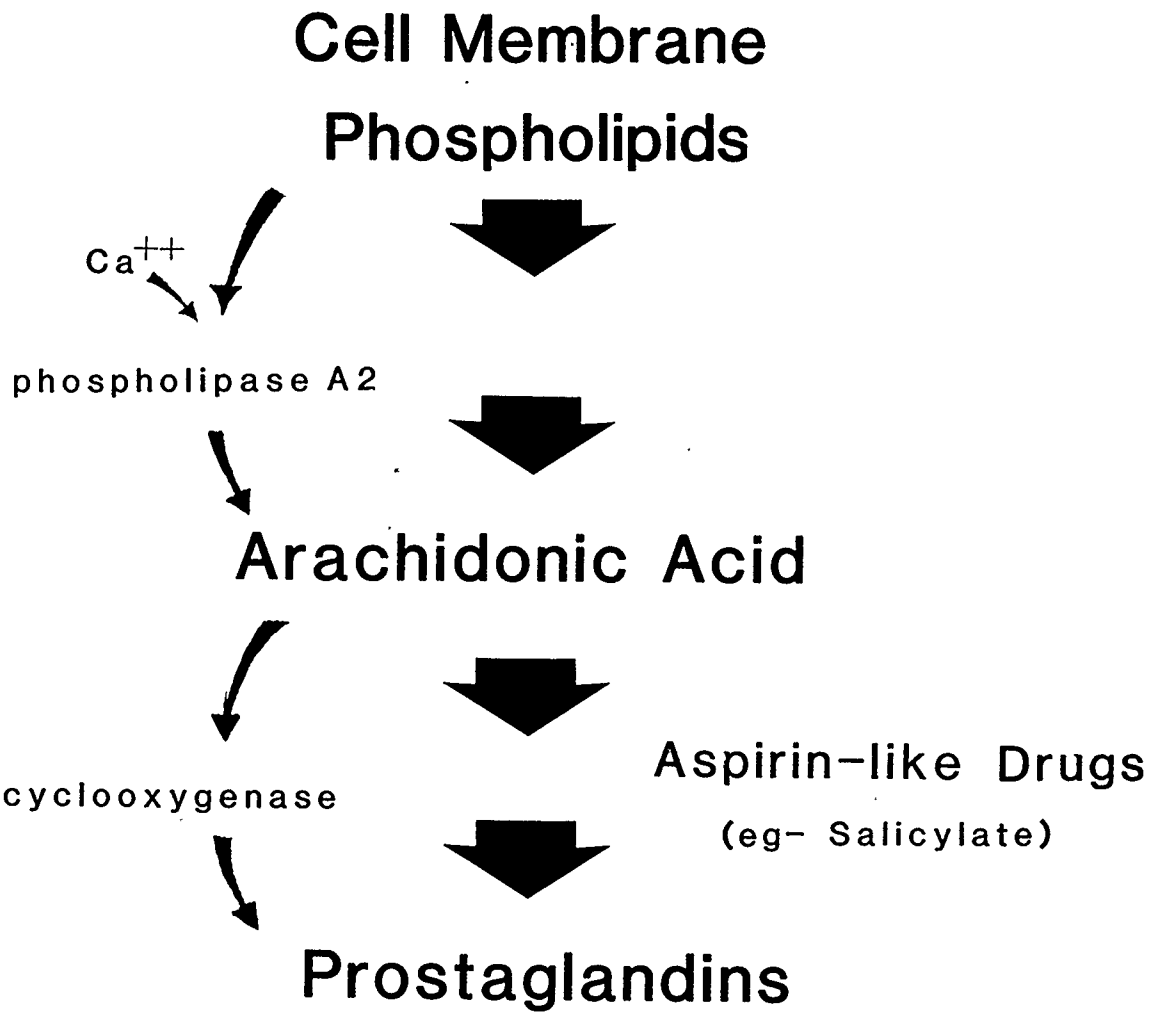
enzymatically cause the release of arachidonic acid from membrane phospholipids (Dinarello, 1984; Coceani et al., 1986; Milton, 1982).

E. Prostaglandins of the E Series

Arachidonic acid can be converted via the cyclooxygenase enzymes: prostaglandin (PG) endoperoxide synthetase, PG hydroperoxidase, endoperoxide reductase and endoperoxide E-isomerase (Harris, 1985) to prostaglandins of the E series (PGE) which have been postulated as the primary mediator of fever (Milton and Wendlandt, 1971; Feldberg and Gupta, 1973; Coceani, 1974). Prostaglandins were discovered in peripheral mammalian tissue in the 1930s but it was not until the 1960s that they were found to be an endogenous neurohumoral agent in bovine brain (Samuelsson, 1964). Research demonstrated that PGE was a potent pyretic when injected icv (Feldberg and Saxena, 1971a; Milton and Wendlandt, 1971) or when injected in the AHPOA (Feldberg and Saxena, 1971b; Stitt, 1973; Veal and Cooper, 1974a). The case for PGE as a primary mediator was much strengthened in 1971 with the discovery that aspirin-like drugs inhibited the synthesis and release of prostaglandins by inhibiting the conversion of arachidonic acid to endoperoxides (Vane, 1971). A schematic diagram of the arachidonic acid cascade is depicted in Figure 1. as well as the step at which the aspirin-like drugs are thought to exert their antipyretic actions. Thus, prostaglandins became the focus of much research and in later years the subject of much

Figure 1.

A schematic diagram of the Arachidonic acid cascade, activation of which leads to the synthesis and release of Prostaglandins of the E series. Key enzymes in the cascade are indicated on the left side of the diagram and the point at which aspirin-like drugs are thought to exert their antipyretic effect is indicated on the right side of the diagram.


Cell Membrane
Phospholipids
Ca++
phospholipase A2
Arachidonic Acid
cyclooxygenase
Aspirin-like Drugs
(eg- Salicylate)
Prostaglandins

controversy. Numerous observations support a mediatory role of PGE in fever although there are several strong arguments to be made against such a role. Both sides of the prostaglandin story will be considered in detail in the following pages since prostaglandins and blockade of PGE hyperthermia by aspirin-like drugs are the focus of the present research.

i. PGE in CSF During Fever

One of the earliest and more convincing pieces of evidence for a role of prostaglandins in fever was that their concentration in the CSF increases during pyrogen-induced fever (Feldberg and Gupta, 1973; Feldberg et al., 1973). That is, when a fever was induced by icv administration of pyrogen, concentrations of PGE in cisternal CSF samples increased from baseline values of 1.3 - 10 ng/ml to 4 - 35 ng/ml. Furthermore, the concentration of PGE was reduced with an ip injection of aspirin-like drugs. In another report following shortly after, Feldberg and his colleagues showed that regardless of the route of pyrogen administration changes in cisternal [PGE] occurred (Feldberg et al., 1973). This work has been repeated using different species and different routes of pyrogen and antipyretic administration (Bernheim et al., 1980; Coceani et al., 1983). Furthermore, it has been shown that other changes in the brain environment do not account for the pyrogen-induced changes in [PGE]. That is, heat exposure, cold exposure, hypothalamic heating or cooling do not affect the levels of ventricular PGE. In one study iv EP resulted in a change in PGE ventricular levels (Bernheim et al., 1980). As well, exogenous overheating does not change

the levels of EP in blood, or, cyclic adenosine monophosphate (cAMP) or PGE in cisternal CSF (Siegert et al., 1976).

The technique used by Feldberg and his colleagues to measure changes in PGE concentration yielded different results for Cranston and colleagues several years later using a different animal species. They showed that a febrile temperature could be maintained at the same time that PGE concentration in cisternal CSF samples was reduced with sodium salicylate (Cranston et al., 1975a). However, the methods of assay used by both of these groups have recently come under review by Coceani and his colleagues who have shown that previous measurements of PG levels may have been artificially high. They have shown that increased levels of PGE can be detected in CSF samples when endotoxin is administered directly into the brain or when precautions are not taken to block leukocyte cyclooxygenase activity in the collection vessel (Coceani et al., 1983). The work of Cranston and his colleagues as well as that of Feldberg observed both of these cautions and so they would not seem to apply. It may be possible to explain the discrepancy on the basis of the site of collection. That is, cisternal CSF may not give an accurate account of PGE synthesis and release in the brain. Particularly in the rabbit which does not have a Foramen of Magendie, cisternal CSF may only be a small part of hypothalamic PGE actually present (Cranston et al., 1976). Furthermore, the hypothalamus may not be the only site in the brain where PGE production and release occurs during fever. PGE very likely originates from other areas of the brain (Dinarello and Bernheim, 1981).

Later investigations did not make use of the rat fundus strip assay for PGE but rather they used a direct radioimmunoassay and found the PGE levels to be much lower. It is important to note that this recent method of PGE assay has indicated that PGE concentration does increase during pyrogen-induced fever (Coceani et al., 1983; Bernheim et al., 1980).

Several points arise from these important papers. Firstly, the method of pyrogen administration has been shown to make a difference in the amount of detectable PGE. That is, intrathecal administration resulted in higher levels detected and a higher number of samples being above detection level than with intravenous administration (Coceani et al., 1983). Secondly, the use of ethanol in the extraction procedure decreased the number of samples with detectable amounts of PGE to very low numbers. Both of the early papers had used ethanol in their bioassay. Thirdly, the site of sample collection seems to affect the ability to detect PGE. That is, cisternal sites of collection did not yield as many samples with detectable amounts of PGE in them as ventricular samples. The collection of CSF from the cisterna magna used by Feldberg and by Cranston may not be a good indicator of PGE release into the brain since this area is anatomically far removed from the POAH or OVLT (organum vasculosum lamina terminalis). Finally, the ability of sodium salicylate, in Cranston's report, to decrease PGE levels while fever continued has now come under objection on the basis of collection site and assay. Results from the more advanced radioimmunoassay for PGE and a ventricular collection site which is closer to the proposed site of action of PGs has shown a positive correlation between [PGE]

increases and pyrogen fever (Cranston et al., 1984; Coceani et al., 1983; Bernheim et al., 1980).

ii. PGE Synthesis Blocked by Aspirin-like Drugs

The prostaglandin involvement as the mediator of fever was reinforced greatly with the discovery by Vane (1971) that aspirin-like drugs block the synthesis and release of prostaglandins. Aspirin-like drugs, such as salicylate, inhibit the cyclooxygenase enzyme thus preventing the conversion of arachidonic acid to endoperoxide intermediates and finally prostaglandins. It is well known that sodium salicylate suppresses pyrogen-induced fever when administered within central areas such as the AHPOA and mid-brain (Cranston and Rawlins, 1972; Avery and Penn, 1974; Schoener and Wang, 1975a; Vaughn et al., 1979). Evidence from electrophysiological studies has shown that sodium salicylate reverses the inhibitory effects of pyrogen on warm-sensitive neurons and the excitatory effects on cold-sensitive neurons (Schoener and Wang, 1974; Hori et al., 1984). These observations support the idea that aspirin-like drugs are antipyretic because of their inhibition of pyrogen action and prostaglandin synthesis and release. This topic will be dealt with at further length in reference to the mechanisms of action of antipyretic drugs.

Recent reviews have argued that the antipyretic action of aspirin-like drugs does not prove that PGE is the sole obligatory mediator of fever (Mitchell et al., 1986) since some antipyretics are known to have effects in addition to the blockade of PG synthesis and release. For instance, indomethacin inhibits cAMP-dependent protein kinase at an

order of magnitude lower than doses which inhibit the cyclooxygenase enzyme (Cantor and Hampton, 1978). Clark and Cumby (1976) have suggested that indomethacin and paracetamol are antipyretic via a central action on EP itself rather than by inhibiting PG synthesis. Additionally there has been a report that aspirin and paracetamol did not inhibit PG synthesis in rabbit brain homogenates at concentrations which reduce fever in vivo (Abdel-Halim et al., 1980). Finally, it has been suggested by Bito and colleagues that aspirin-like drugs may interfere with the transport of prostaglandins in the brain as well as with their synthesis and release (Bito and Salvador, 1976). While the ability of aspirin-like drugs to block PGE synthesis and release is well established, it cannot be denied that other drug actions may be occurring which are responsible for antipyresis.

iii. PGE Synthesis Activated by Pyrogens

If prostaglandins were to be a primary mediator of fever it would be important to show that their synthesis is activated by pyrogenic substances. Ziel and Krupp (1976) showed that the biotransformation of arachidonic acid to prostaglandins in cortical cells in vitro is specifically augmented by EP to a degree which is concentration dependent. It is interesting to note that this effect of EP was inhibited by aspirin-like drugs. Recently Bernheim and Dinarello (1985) have shown that PGE_2 production can be increased by leukocytic pyrogen in cultured hypothalamic cells. Thus it would seem that, at least in cultured cells of the brain, pyrogens do indeed cause the synthesis and release of prostaglandins. There are several other conditions which have been reported to effect prostaglandin synthesis and/or release

including: the availability of arachidonic acid which depends on the activity of phospholipase A2, a calcium dependent, membrane enzyme; PGE_2 release from hypothalamic neurons of the median eminence depends on calcium availability (Ojeda and Negro-Vilar, 1984); and the synthesis of PGE_2 in the adenohypophysis can be influenced by adrenocorticotropin and B-endorphin release (Knepel et al., 1985). The point is that there are many physiological neurohumors and numerous physiological events, such as ion concentrations and release of neuroactive substances, which may influence the synthesis and release of PGE in the brain as well in any tissue of the body likewise affected. As well as being effected by these events and substances, PGEs themselves have complex and diverse actions on transmission in pathways of the brain, spinal cord and on single neurons (Wolfe, 1978).

iv. Electrophysiological Effects of PGE

Electrophysiological studies have not strengthened the scientific evidence either for or against the role of PGE as the mediator of fever. One group of investigators (Ford, 1974; Stitt and Hardy, 1975) applied PGE to AHPOA thermosensitive neurons microiontophoretically and found only a rather small number of cells, usually cold sensitive, were excited by PGE. A second group, however, found that a majority of thermosensitive cells were responsive to brief applications of PGE (Schoener and Wang, 1976; Jell and Sweatman, 1977; Gordon and Heath, 1980). Further to this, PGE application in preoptic, septal and arcuate areas was excitatory although onset of excitation had various time courses (Poulain and Carette, 1974). Nevertheless, a comprehensive review of the electrophysiological effects of PGE by Eisenman (1982)

indicated that 75% of warm-sensitive neurons and 50% of cold-sensitive do not respond to PGE application. Either PGE action on neurons is not at all like that of EP (Mitchell et al., 1986) or the site of action of PGE is presynaptic to the thermosensitive neurons of the AHPOA and therefore iontophoresis would not effect the neurons (Veale et al., 1977).

v. PGE is Pyretic in Most Species

Contrary to the many species-specific effects of the thermoregulatory neurotransmitters, 5 HT and NE, PGs as well as EP have consistent thermogenic effects across the species of mammals tested. PGE produces hyperthermia in cats, rats, sheep, squirrel and rhesus monkeys and guinea pigs. In nearly every species tested, vertebrate and invertebrate, PGE has caused physiological and/or behavioral responses like those occurring during a fever. Exceptions to this statement include: the echidna, in which icv administration of PGE causes a drop in body temperature (Baird et al., 1974); young animals of certain species such as the chick (Artunkal et al., 1977) although mature chickens do respond to PGE with fever (Pittman et al., 1975a); and the newborn lamb which does not respond to icv PGE_1 (Pittman et al., 1975b) although in some hands the sheep is capable of generating fever to icv PGE (Hales et al., 1973; Bligh and Milton, 1973). Pittman and colleagues conducted an anatomical search in sheep including lateral, anterior and posterior hypothalamic regions and found no temperature responses to PGE injection (Pittman et al., 1975b). It has also been reported that icv PGE has no effect on the body temperature of the goat (Leskell, 1978). These exceptions may indicate an immature fever

production pathway (in young) although the lambs were able to respond to iv BP or EP. Alternatively, they may indicate a lack of PGE sensitivity in these mammals which argues against a mediatory role for PGs in fever.

vi. PGE Causes Fever at the Same Sites as EP

When the dose-response curves for PGE_1 in several species are compared, the slopes of all the curves are strikingly similar (Stitt, 1986). The marked increase in body temperature upon PGE administration occurs within the same brain sites where EP is found to be pyrogenic, that is, the cerebral ventricles (Feldberg and Saxens, 1971a) and the AHPOA (Feldberg and Saxens, 1971b). Like EP, PGE was found to lack pyrogenicity in brain sites such as the posterior hypothalamus (Veale and Cooper, 1975). A search for extrahypothalamic sites began early on (Rosendorff and Mooney, 1971) and later, extensive mapping studies revealed other PGE sensitive areas of the brain (Williams et al., 1977). Recently discrete regions of the septal area have been found to be sensitive to the effects of several pyretic and antipyretic substances. Prostaglandins are pyretic when administered within the ventral septal area of the rat (Ruwe et al., 1985a), as is EP in the rabbit (Naylor et al., 1986). Another discrete region of the brain has recently been shown to have very sensitive to the pyrogenic effects of PGE. The organum vasculosum lamina terminalis (OVLT) is, in fact, more responsive to the hyperthermic effects of PGE then the AHPOA is (Stitt, 1986). In sum, the ability of PGE to cause marked increases in temperature at the same loci where EP is pyretic has been taken as further evidence that PGE could serve as the link in the production of pyrogen-induced fever.

vii. PGE Doses- High or Low?

It has been suggested that icv injections of PGE are made against a diffusion gradient so in order to affect the AHPOA the doses must be high and not physiological (Mitchell et al., 1986). These authors have contested that doses given into hypothalamic tissue although they are lower than icv doses are still too high. For the sake of argument, Stitt (1986) published a review which points up the fact that there is no appreciable difference between dose-responses of PGE given icv or intrahypothalamic (ih) indicating that the AHPOA may not be the site of PGE action at all. The sensitivity of the OVLT to PGE however is very high. That is, the slope of the dose-response curve for PGE in the OVLT is more than twice that of the AHPOA and the dose-response threshold for OVLT PGE fevers is five times less than for AHPOA PGE fevers.

viii. Lesion Studies

Lesioning the prostaglandin sensitive AHPOA in rabbits resulted in the inability to respond to PGE (icv) although febrile responses to EP (icv) were still intact (Veale et al., 1977). This suggested that there was a region of brain still intact which was sensitive to pyrogens and that perhaps PGE was not necessary for fever.

ix. Time to Fever Onset

Further evidence for PGE concerns the onset of increases in temperature caused by PGE as compared to EP. PGE-induced increases are observed almost immediately following central administration, whereas the latency of onset of the febrile response to EP takes as long as 7 -

20 minutes (Stitt and Hardy, 1972; Stitt, 1973). This is supportive of PGE as mediator of pyrogen-induced fevers (Veale and Cooper, 1973).

x. PGE and Set-Point Changes

Experiments showing that PGE_1 appeared to raise the set-point temperature of animals, just as pyrogens did, have provided evidence for PGE as mediator of fever. For example, Stitt (1973) found that rabbits, and Veale and Wishaw (1976) found that rats, responded to PGE1 in the AHPOA in a dose-dependent manner regardless of the ambient temperature. Similar observations have been made with regard to iv and central injections of EP in the rabbit (Cooper, 1972a). Thus, PGs would cause a rise in temperature through an increase in heat production and/or a decrease in heat loss (Stitt, 1973). This suggests that PGE, like pyrogen, has febrile effects resulting from a resetting of the thermoregulatory set-point to a new and higher level.

xi. Pharmacological Experiments Using PG antagonists

Yet another line of evidence was provided by experiments which applied conjugated PGE_1 antibody to the AHPOA of rabbits. Slow infusion of the antibody caused a slow, long-lasting fever although 1-10 days later EP administration had very little, if any, effect on temperature. This gave support to the idea that EP may increase the release of PGE in the AHPOA (Veale and Cooper, 1974b).

There are considerable numbers of reports in the literature which, having made use of antagonists and protein inhibitors, would argue the work with antibody just mentioned. A prostaglandin antagonist, SC

19220, was first injected ip into rabbits in order to antagonize a fever, however, it did not decrease fever and had severe side effects (Sanner, 1974). SC 19220, as well as HR 546, administered icv blocked PGE-induced hyperthermia but did not effect the latency, rise or magnitude of fever induced by icv EP (Cranston et al., 1976). These authors suggested that the antagonist might selectively antagonize the passage of PGE but not EP through the ependyma of the third ventricle to the AHPOA assuming the AHPOA is the sole site of action for the hyperthermic effects of exogenous PGs and pyrogen (Cranston et al., 1976).

The effect of the PGE antagonist on fever evoked by arachidonic acid (AA) has yielded various results. By itself, AA icv causes fever in cats (Clark and Cumby, 1976); rats (Splawinski et al., 1974) and in rabbits (Laburn et al., 1977). The latter group showed that AA fever can be inhibited by icv indomethacin indicating that the hyperthermic effects of AA are due to metabolites and not AA itself. On the other hand, they reported PGE antagonists (SC 19220 and HR 546) to be ineffective "except for the first few minutes" against AA-induced fevers. That is, the PGE antagonists did attenuate AA fever for approximately 30 minutes as indicated by the data. Thus, the authors postulate two pyrogenic derivatives, one of which is PGE and the other undetermined. The unknown derivative appears not to be a leukotriene (Cranston et al., 1984) and other prostanoids have not been investigated adequately as they are unstable compounds and difficult to utilize in such experiments.

xii. Mediators Other Than PGE

Numerous experiments have attempted to identify another mediator of fever. It has been suggested that one of the several metabolic products of arachidonic acid might play the role of febrile mediator (Cranston et al., 1978, 1983; Townsend et al., 1984). The results of experiments using protein synthesis inhibitors and inhibitors of phospholipase A2 have not revealed, as yet, which metabolites are linked to the mediation of fever.

xiii. Summary

It would appear as though the arguments for and against PGE as mediator have gone full circle beginning in the 1970s with the pyrogenity of PGE itself and its presence in CSF (which was disputed soon after) to the present day with PGE pyrogenicity spanning across a great many species and its presence in the CSF being directly correlated with rising and stabilized febrile temperatures. Clearly a more specific assay with a lower threshold has helped to clarify previous discrepancies (Townsend et al., 1984). Many investigators, in order to justify and envelop the enormous amount of data on PGE, have endeavored to put forth a coherent hypothesis. It has been postulated that PGE may have a two part role in fever. Firstly, to mediate the very early thermal events in fever occurring in the first 30 minutes or so and then secondly, to sensitize thermosensitive neurons in the brain to EP, endogenous neurotransmitters or other such substances (Mitchell et al., 1986). Alternatively, it has been hypothesized that the AHPOA is not the primary site of action of febrile substance, but that the OVLT is an area fulfilling many of the criteria for such a role (Stitt, 1986).

Many of the contradictory pieces of evidence can be understood in the context of either of these hypotheses. Suffice it to say that the pyrogenic abilities of PGE are well established and there is a wealth of information supporting the view of PGE as a primary mediator of fever. As with most physiological investigations there has been evidence which does not fit with such a role and disputes the necessary involvement of PGE in the pathogenesis of fever.

F. Antipyretics

i. Exogenous Antipyretics

Historical Review

The therapeutic benefits of the willow bark, salix alba, have been known since the classical age of Hippocrates and Galen. The popularity of the willow however has not always been as strong as it is today. In 1639 an Augustine monk from Spain introduced cinchona bark taken from "the fever tree" as a potent and effective cure for fever. The cinchona bark, the effective component of which is quinine, was a common therapeutic agent demonstrated by its inclusion in the London Pharmacopoeia in 1677 up until the time of the Napoleonic war when its availability was restricted (cf Rollo, 1975). The bark of the willow came back into fashion in the late 1700s due to a convincing communication written to the Royal Society by Reverend Edmund Stone in 1763. The Reverend made a compelling case for the virtues of the willow as an antipyretic (cf Milton, 1982). Throughout the Middle Ages the

bark, sap and ashes of salix alba were used for medicinal purposes (cf Haas, 1983).

The active principle of salix alba is the glycoside salicin, which was first extracted by Leroux in 1827. In 1838 Pira used salicin to procure salicylic acid while six years later Cahours prepared salicylic acid from oil of wintergreen (Woodbury and Fingl, 1975). In 1860 the acid was synthesized from phenol by Kolbe and Lautemann (Hass, 1983). Sodium salicylate was first employed by Buss as an antipyretic for rheumatic fever in 1875 (Haas, 1983). The unpleasant side effects of sodium salicylate, such as gastrointestinal irritation and the bitter taste, led to a search for other more appealing derivatives. This resulted in the lucrative discovery of the ever-popular acetylsalicylic acid or aspirin and its introduction into medicine by Dresser in 1899. Bayer began marketing aspirin immediately and it has been used all over the world as an analgesic, anti-inflammatory and antipyretic ever since.

Various other antipyretics were discovered early on as well. The pyrazalone, antipyrine, discovered by Knorr in 1883 was used for its potent anti-inflammatory nature until the 1970s when it was banned from use in many countries because of its toxic side effects. Acetanilid was accidently discovered as an antipyretic and then used therapeutically by Cahn and Hepp in 1886. In vitro it is oxidized to aminophenol, a finding which led to the synthesis of phenacetin and its testing in 1887 and 1888 and continued use until around 1953 when adverse effects were reported (Hass, 1983). Acetaminophen was first synthesized in 1878 although not introduced into clinical practise until 1893. Its use

increased dramatically after 1943 when Brodie and Axelrod (1949) discovered that it was the active analgesic form of phenacetin. In 1955 acetaminophen was marketed as Tylenol and has been the best-selling analgesic since then in the U.S. (Haas, 1983). Indomethacin was the result of a search for drugs with anti-inflammatory abilities and was introduced medically in 1963 for the treatment of rheumatoid arthritis. It readily demonstrated potent antipyretic effects as well, although chronic use as a general analgesic-antipyretic is not well tolerated by patients (Woodbury and Fingl, 1975). One very recent antipyretic is known as AD-1590, a proprionic acid substance which has been used in experimental antipyresis. Its potency as an antipyretic exceeds indomethacin, aspirin, sodium salicylate and several others (Nakamura et al., 1984). The chemical structures of several common antipyretic drugs are displayed in Figure 2.

Experimental Antipyresis

Under experimental conditions salicylate antipyresis has been studied in the laboratory for more than a century. In 1875 Zimmerman showed that fever induced by iv injection of putrid fluids could be attenuated by oral salicylic acid in doses of 100 - 500 mg (cf Grundmann, 1969) Experiments testing one antipyretic and another continued through the 1800s and have grown in scope and numbers throughout the 1900s. Only a select few will be highlighted at this point in an effort to introduce basic principles and concepts regarding the action of antipyretic drugs. In 1913 Barbour and Wing applied antipyretics directly to the corpus striatum of rabbits and observed a reduction in fever induced by heat puncture (Barbour and Wing, 1913).

These and similar experiments served as the first indication that the antipyretic effect was due to central action of the drug.

In 1921 Henry Barbour observed that antipyretics did not effect normal body temperatures of febrile subjects and thus he provided us with the definition currently used. Antipyretics are substances which reduce the temperature in febrile and similar states but not in normal conditions, unless the dosage be excessive (Barbour, 1921). Rosendorff and Cranston (1968) confirmed this when they showed that salicylate reduced naturally occurring fevers in humans but did not change the normal body temperature of volunteer afebrile humans.

In 1943, monkeys made febrile with subcutaneous injection of yeast were given oral aspirin which reduced the fever and was accompanied by hydraemia and sweating (Guerra and Barbour, 1943). The following year in different monkeys, bilateral electrolytic lesions of the anterior hypothalamus did not prevent the yeast fever and aspirin antipyresis from occurring although there was a reduction in sweating (Guerra and Brobeck, 1944). Even though the antipyreic effect was unaffected, Guerra concluded (1944) that aspirin altered AH sensitivity so that temperature was reduced by heat loss initiated by haemodilution. These experiments demonstrated that even though fever could still be produced without the anterior hypothalamus intact, thermoregulatory effectors were adversely affected.

Mode of Action

With the considerable research on the pathogenesis of fever and its antagonism by antipyretic drugs, it became important to determine at which point in the fever pathway antipyretics were acting. Initially it was thought that the salicylates might be having their effect on fever by decreasing the yield of leukocyte pyrogen (EP) from white cells of the RES (Gander et al., 1967). In Gander's experiments salicylate did not reduce intravenous EP fever while it did reduce BP fever, thus a peripheral action was postulated. However other investigators soon showed that salicylate could reduce fevers caused by EP injection iv (Cranston et al., 1970a) in a dose-dependent manner. Furthermore, another antipyretic, 4-acetamidophenol, was shown to reduce fever caused by icv pyrogen (Milton and Wendlandt, 1968). These observations and many others like them have established that antipyretics do not effect pyrogen-cell interaction or the release of EP (Bennett and Beeson, 1953; Bennett, 1956; Lin and Chai, 1972).

Another possible peripheral action of antipyretics was the inactivation of EP while it is circulating in the blood. However experiments have shown that the incubation of EP with antipyretics does not inactivate the EP or effect its potency (Grundmann, 1969; Clark and Cumby, 1975). The question as to how the aspirin-like drugs restored the elevated set-point of fever toward normal still remained. Several suggestions were made:

1. Antipyretics might alter the rate at which EP entered the brain through the blood brain barrier (Cooper et al., 1968).

2. Antipyretics might influence (accelerate) the excretion or destruction of EP within the brain (Cooper, 1972a)
3. Antipyretics might act on neurons or competitively interfere with EP action on the cell surface of thermoregulatory cells (Wit and Wang, 1968; Rawlins et al., 1971)
4. Antipyretics might effect the thermoregulatory effectors such that heat loss was increased or heat production was decreased (Satinoff, 1972).

It is unlikely that antipyretics act by preventing the passage of EP into the brain for several reasons. Firstly, antipyretics act effectively to block pyrogen fever when they are administered within the AHPOA or the ventricular system (Cranston et al., 1970a; Cranston and Rawlins, 1972; Avery and Penn, 1974; Vaughn et al., 1979). Secondly, salicylate microinjected into the AHPOA reverses the pyrogen-induced inhibition of firing on warm-sensitive cells and excitation of cold-sensitive cells (Schoener and Wang, 1975a; Nakashima et al., 1985). Thirdly, fever due to centrally injected EP has been suppressed by antipyretics administered peripherally (Cranston et al., 1970b; Lin and Chai, 1972; Clark and Cumby, 1975). Fourthly, it is not at all clear that EP gets into the brain (Stitt, 1986) although the OVLT which is very near to the AHPOA and does not have a blood brain barrier may be a likely site (Mashburn et al., 1983; Dinarello, 1984) if one exists. Although it could not be ruled out entirely that antipyretics block the entry of EP into the brain, it appeared more likely that antipyretic action was mediated at least partly by antagonizing the effects of EP within the brain.

The bulk of the evidence concerning the action of antipyretic drugs has suggested that antipyretics act on central neurons. The variety of ways in which a direct action could come about are listed below and include those from Kasting et al (1982a).

1. Inhibition of prostaglandin synthesis or other arachidonic acid metabolites and/or inhibition of prostaglandin transport systems within the brain
2. Competitive antagonism with EP or like substance at a specific EP receptor
3. Indirect antagonism of ionic mechanisms controlling set-point in the posterior hypothalamus
4. Indirect antagonism by changing the membrane characteristics of thermoregulatory cells
5. Indirect antagonism through the release of an endogenous antipyretic substance

1. The discovery that aspirin-like drugs blocked the synthesis and release of prostaglandins (Vane, 1971) was originally made in lung homogenate, but such an action has been observed subsequently in numerous tissues including those of the central nervous system (Vane, 1978). That aspirin-like drugs have their effects in the CNS by direct effect on PGE has been supported by a number of observations: Peripheral administration of aspirin-like drugs suppressed pyrogen-induced fevers at the same time that the PGE-like activity in cisternal CSF samples was lowered (Feldberg et al., 1973); peripherally administered aspirin-like drugs suppressed pyrogen-induced fevers, but did not block PGE-induced

hyperthermia (Milton and Wendlandt, 1971; Avery and Penn, 1974; Schoener and Wang, 1974; Woolf et al., 1975; Clark and Cumby, 1975); and within the AHPOA, a significant correlation between the magnitude of a PGE-induced fever and the magnitude of antipyresis brought about by salicylate has been shown (Vaughn et al., 1979). Thus there is considerable evidence supporting the mode of antipyretic action of aspirin-like drugs to be through the inhibition of the cyclo-oxygenase enzyme needed for production of prostaglandin E.

There is also evidence that antipyretic drugs inhibit the transport, as well as the synthesis, of prostaglandins (Wallenstein and Bito, 1978). It has been shown that indomethacin, aspirin and salicylate inhibit the biotransport of labelled PGs from ventricular ECF to the choroid plexus of rabbits (Bito and Salvador, 1976). The ability of aspirin-like drugs to modify the disposition and distribution of endogenous and exogenous prostaglandins (Bito and Salvador, 1976; DiBenedetto and Bito, 1986) may help to explain a part of their anitipyretic nature.

2. Antipyretics may act however in an entirely different manner within the CNS, for example, to compete with EP (or like substance) for a receptor on the neurons. This has been indicated by a parallel shift to the right of the log-dose response curve for EP fever during indomethacin, acetaminophen and salicylate treatment (Clark and Coldwell, 1972). Shifts of the log-dose response curve have been verified under other conditions (Lin and Chai, 1972; Clark and Cumby, 1975). It has been suggested that since the chemical structures of the

antipyretics are so dissimilar from EP's structure it is likely that the antagonism was a case of allosteric inhibition at the receptor (Clark and Coldwell, 1972) rather than direct competition for the receptor. A further challenge to the idea of direct antagonism was provided by experiments in which injections of EP to one side of the hypothalamus and acetylsalicylate (ASA) to the contralteral side were made. Neurons which had increased their firing rate upon exposure to EP, subsequently reduced their rate of firing after the ASA was applied (Schoener and Wang, 1975b). Considering the low solubility of ASA it seems unlikely that it acts in fever by directly competing for the same receptor as EP.

3. The third suggestion concerning the action of antipyretics on central neurons involved an indirect antagonism of ionic mechanisms maintaining the set-point. Any effect on the Na^{+}/Ca^{++} ratio within the posterior hypothalamus would have to be an indirect one since the site of action of antipyretics has pointed to the anterior hypothalamus, midbrain or other extrahypothalamic areas of the brain. Regardless, there have been several reports of antipyretics altering ion permeabilities, and ion fluxes in central neurons (Cameron, 1968; Myers, 1974).

4. Along the same line, the fourth suggestion as to the central action of antipyretics on neurons is that of an indirect antagonism resulting from alterations of membrane characteristics. Large central neurons in the mollusk navanax inermis responded to salicylate application with an immediate, dose-dependent hyperpolarization of the resting membrane potential as well as a decrease in the cell's input resistance (Barker

and Levitan, 1971). The ionic mechanisms involved included an increased K^+ permeability and a decreased Cl^- permeability. The effects of salicylate, which were reversible, occurred very quickly suggesting direct alteration of membrane characteristics rather than the more time-consuming metabolic effect of salicylate. Similar effects have been demonstrated on: AHPOA and septal neurons in the rat (Beckman and Rozkowska-Ruttiman, 1974); on temperature-sensitive human red blood cells (Weith, 1970) where salicylate decreased Cl^- permeability; on the exchange of K^+ between blood and CSF, which increased upon exposure to salicylate (Cameron, 1968). Effects such as those mentioned above would serve to reduce the input to and the output from a neuron, that is, to render the cell somewhat insensitive.

There is a high correlation between the ability of an antipyretic compound to alter membrane potential and ion conductance and its lipid solubility (Levitan and Barker, 1972). Thus, one would predict that aspirin would be less active and indomethacin would be much more active on neurons than salicylate.

Recently it has been suggested that EP acts like a Ca^{++} ionophore so that rapid accumulation of intracellular Ca^{++} induces changes in membrane function such as activation of phospholipase A2 and the arachidonic acid cascade (Dinarello, 1984). It might be that instead of, or in addition to altering K^+ and Cl^- conductances, antipyretics might alter Ca^{++} conductance of the membrane so as to negate the effect of the EP. Needless to say there is much that needs to be determined in

terms of the effect the antipyretic drugs have on the membrane properties of central neuronal membranes.

5. The fifth suggestion which was outlined as a possible mechanism of central action of antipyretics was the indirect antagonism of fever through the release of an endogenous antipyretic. The existence of such an endogenously-occurring peptide antipyretic, arginine vasopressin, has been established (Kasting et al., 1978a; Cooper et al., 1979; Veale et al., 1984) and its release, as measured in tissue perfusates, correlates in a negative fashion with temperature increases (Cooper et al., 1979; Kasting, 1980a). Consistent with the idea that exogenous antipyretics may lead to the release of endogenous antipyretics, Brooks et al., (1984) have shown that centrally administered indomethacin enhances the vasopressin response to volume depletion. Even though it was peripheral release that was measured, it sparks interest as to whether central release might also be enhanced, since others have shown that increasing the blood osmolarity in the rabbit is associated with increases in both peripheral release and central release of AVP. Perhaps administration of exogenous antipyretics serves as a perturbation of homeostatic processes thus causing the release of the neuropeptide (Kasting et al., 1982b). In considering the possible actions of antipyretics on central neurons it is quite possible that an interaction of the mechanisms mentioned above would bring about the reduction of a fever.

Alternatively, the mechanism of action for antipyretic drugs may not involve direct effects on central neurones at all. The possibility that antipyretics reduce fever by interfering with the thermoregulatory

effector mechanisms of the body has been suggested. There have been some reports in the literature of antipyretics causing hypothermia which would account for fever reduction (Satinoff, 1972; Feldberg and Saxena, 1974; Polk and Lipton, 1975). Experiments on cold-exposed female rats showed that intraperitoneal (ip) salicylate significantly reduced normal body temperature in a dose-dependent manner (Satinoff, 1972). As well, rats at room temperature also became hypothermic due to ip salicylate (Satinoff, 1972; Polk and Lipton, 1975) and in one study to paracetamol and indomethacin (Feldberg and Saxena, 1974). All three groups used rats and intraperitoneal injections of the antipyretics.

In sharp contrast, are the majority of reports demonstrating very little effect of antipyretic drugs on body temperature. Following antipyretic administration (orally, iv, ip, icv, iontophoretically) normal body temperatures have been reported for humans (Rosendorff and Cranston, 1968); monkeys (Guerra and Barbour, 1943); cats (Wit and Wang, 1968; Cranston et al., 1975b; Schoener and Wang, 1975a); rabbits (Cameron and Semple, 1968; Cranston et al., 1979a, 1970b; Woolf et al., 1975; Pittman et al., 1976); and rats (Avery and Penn, 1974; Scales and Kluger, 1986). It would appear that rats may be susceptible to ip administration of antipyretics whereas other routes of administration do not affect normal body temperatures of rats or other species.

Biochemical Characteristics

In terms of antipyretic therapy it is important to understand the action of the antipyretics on living tissues. Non-steroidal anti-inflammatory drugs or NSAIDs are acids which inhibit the biosynthesis of prostaglandins and several are stereospecific (Gryglewski, 1974). Structure-activity studies have shown that salicylates generally act by virtue of their salicylic acid content and substitutions on the carboxyl or hydroxyl groups only change the potency or toxicity of the drug. The hydroxyl group in the ortho position is important for the action of salicylate (Woodbury and Fingl, 1975). The chemical structures of several of the more common antipyretics are shown in Figure 2.

Most NSAIDs inhibit the enzyme prostaglandin synthetase in a time-dependent, dose-dependent and irreversible manner. The mechanism of inhibition is complex however and may differ for various NSAIDs (Gryglewski, 1974). Packham (1982) has listed ten or more actions for both aspirin and sodium salicylate indicating the reduction of fever as only one. Along with the NSAIDs there are other groups of compounds which inhibit PG synthetase including: some non-narcotic analgesics, e.g., 4-acetamidophenol (Flower and Vane, 1972); some fatty acids e.g., eicosa-8 cis, 12 trans, 14 cis-trienoic acid (Nugteren, 1970); and some metal ions, e.g., Cu^{++}, Ca^{++}, Zn^{++}, Ag^{+} and Au^{+} (Deby et al., 1973). The most commonly used antipyretics however, are the salicylic acid derivatives acetaminophen and indomethacin which are all taken orally (Kasting et al., 1982).

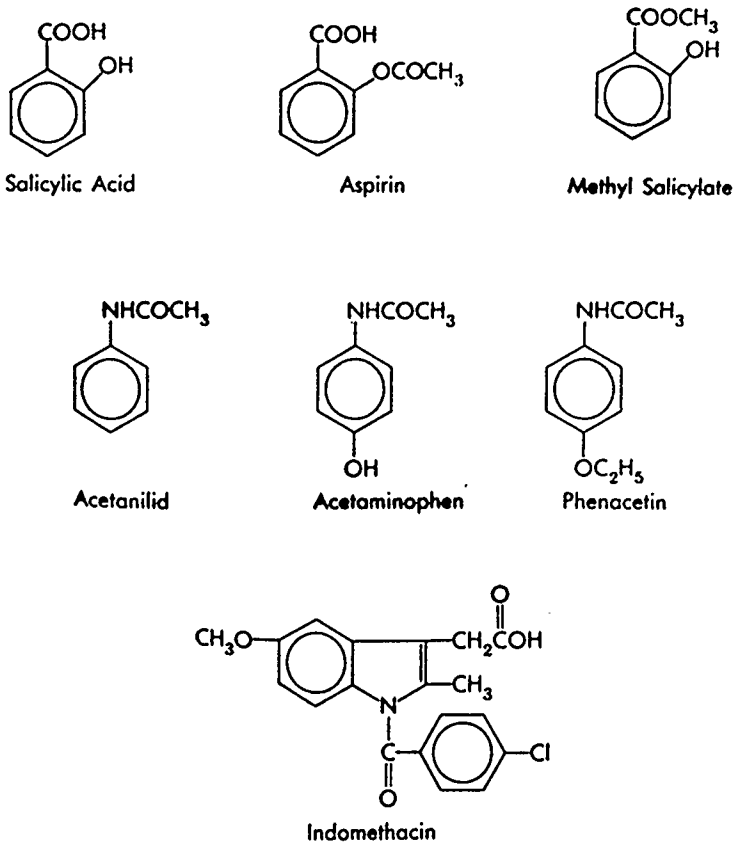


FIG. 2

The chemical structures of the salicylates and other common antipyretic agents (Flower et al, 1985).

Metabolism and Therapeutic Effects

Aspirin in the body is almost completely hydrolyzed to salicylic acid (SA) by esterases (Thiessen, 1982). SA in turn is eliminated by renal excretion or by one (or more) of four biotransformation pathways. The two major pathways for elimination of SA involve conversion to salicyluric acid and/or salicyl phenolic glucuronide (Otto, 1983). The existence of five pathways explains the range of amounts of metabolite reported in the literature. That is, small doses do not saturate the major pathways and therefore the half-life of the drug is short (2.4 - 2.9 hrs) whereas large doses saturate the major pathways and increase the half-life considerably to between 19 and 22 hrs (Otto, 1983; Thiessen, 1982).

The non-lipid soluble antipyretics such as the salicylates do not affect normal body temperature at therapeutic doses (Rosendorff and Cranston, 1968) whereas they lower body temperature during fever through a central effect on the set-point. They do not inhibit heat production, but enhance heat loss through increased blood flow and sweating. Salicylates also relieve low intensity pain through actions both in the periphery and the CNS (Woodbury and Fingl, 1975). The concentration of salicylate in the rabbit following intravenous administration of the standard antipyretic dose (300 mg) varies from 30-45 mg/100 ml in the plasma to 2.4 - 2.7 mg/100 ml in the CSF (Grundmann, 1969). Oddly enough, neither the concentration of salicylate in the CSF (Grundmann, 1969) nor the regional distribution of salicylate in discrete brain areas (Rawlins et al., 1973) varies for febrile and afebrile rabbits. Nevertheless, the concentrations which are achieved seem to be

sufficient to inhibit PGE synthesis during antipyresis (Rawlins et al., 1973). When the dosage of salicylate is too high there are a number of nasty and toxic effects which arise. Some of the toxic effects of salicylate poisoning include: nausea, vomiting, convulsions, confusion, dizziness, stupor, tinnitus, high-tone deafness, delerium, psychosis, and coma (Woodbury and Fingl, 1975). Overdose of aspirin-like drugs can also lead to paradoxical hyperthermia. This is due to the uncoupling of oxidative phosphorylation in the tissues so that the energy normally stored in high-energy compounds is dissipated by an increase in body temperature (Segar and Holliday, 1958). Furthermore, salicylate intoxication interferes with normal cooling mechanisms and the accompanying dehydration worsens the hyperthermia (Temple, 1978).

Acetaminophen is an effective alternative to Aspirin for its antipyretic and analgesic uses. The antipyretic activity of the drug resides in the aminobenzene structure. It is absorbed rapidly and almost completely from the stomach and reaches peak levels in the plasma in 0.5 - 1.0 hrs. The majority of acetaminophen is excreated in the urine after conjugation with glucuronic acid in the liver (Otto, 1983). Side effects which may occur include skin rashes and drug fever or in the most serious cases of overdosage, renal damage and hepatic necrosis. In recommended doses however, acetaminophen and phenacetin are well tolerated (Woodbury and Fingl, 1975).

Indomethacin also inhibits the biosynthesis of prostaglandins and is an effective antipyretic and anti-inflammatory drug. It is rapidly and almost completely absorbed from the stomach. Ninety percent of

indomethacin is bound to plasma proteins and plasma concentrations peak after 3 hours or so (Woodbury and Fingl, 1975). Most of the drug is converted to inactive metabolites and 10-20 percent is eliminated unchanged. The free and conjugated metabolites are excreated in the urine, bile and feces (Duggan et al., 1972). A relatively large number of people experience untoward side effects from normal doses of indomethacin including: various gastrointestinal problems, peptic ulcers, severe headache, dizziness, vertigo, confusion and several other CNS abnormalities, blood related damage, and hypersensitivity reactions.

Clearly it would be important to consider such side effects and toxicity when using these antipyretic compounds. In considering the numerous side effects and other actions that the NSAIDs have it seems apparent that they do not exert all of their effects through the inhibition of prostaglandin synthesis. For instance, it is possible that there are other enzymes involved in the production of fever which are inhibited by the NSAIDs that have not been discovered as yet (Packham, 1982).

ii. Endogenous Antipyretics

Discovery of Arginine Vasopressin

Endogenous steroid substances such as cortisone and cortisol have been recognized as antipyretic for some time (Atkins et al., 1955; Chowers et al., 1968). The steroids have been used therapeutically for many years although their use as antipyretics is limited because of severe side effects (Azarnoff, 1975). The discovery of an endogenously

occurring peptide antipyretic was made in 1978 when it was found that near-term ewes did not get fevers (Kasting et al., 1978a, 1978b). The blood plasma level of arginine vasopressin (AVP) correlated negatively with the magnitude of the febrile response to endotoxin (iv).

Site of Action

To determine if the antipyretic effect of AVP was due to a central action, adult non-pregnant sheep were implanted stereotaxically with cannulae so that various brain sites could be push-pull perfused with AVP solutions. AVP-induced antipyresis was limited to the septal region of the brain and the amount of AVP recovered in perfusates from this region correlated negatively with increased temperature (Kasting et al., 1979a; Cooper et al., 1979). The antipyretic effects of AVP were not produced upon administration intravenously (Cooper et al., 1979). Quite the opposite response was observed when AVP was given icv, that is, a hypothermic response was seen along with disturbances in motor behavior (Kasting et al., 1980b).

Indirect evidence that central AVP and not peripheral AVP is responsible for the antipyretic effect has been collected. It has been shown that hemorrhage-induced release of AVP into plasma and CSF occurs concurrently with a reduction in the febrile response (Kasting et al., 1981). Other indirect evidence has shown that while heat stress and prolonged moderate heat exposure induced changes in the concentration of AVP in the septal area, plasma AVP levels did not change (Epstein et al., 1984). In the same animals dehydration and heat stress together

did change plasma AVP levels indicating a dissociation between the effects of temperature and osmotic stimuli.

Very recently, arginine vasopressin has been found to be antipyretic when administered within the ventral septal area (VSA) of rabbits (Ruwe et al., 1983; Naylor et al., 1985); rats (Ruwe et al., 1985a); guinea pigs (Merker et al., 1980) and cats (Ruwe et al., 1986; Naylor et al., 1986a). Many other differences in central and systemic responses have been reported such that it is quite plausible to suggest that there is a separate and independent cerebral peptide axis (Morris et al., 1984).

AVP is a nonapeptide synthesized in neuronal perikarya located in the hypothalamus. It was not known whether AVP was acting as a hormone, a releasing factor, or a neuromodulator (Kasting et al., 1982b), although some investigators thought that AVP fulfilled many of the criteria for a neurotransmitter of the brain (Buijs, 1983). Needless to say the discovery of this antipyretic peptide has led to investigations as of its neuroanatomical distribution, mode of action and pharmacological nature.

Neuroanatomical Distribution

In order to earn the title as a neuromodulator of the febrile process, AVP would have to exist within the brain areas where it is thought to exert its antipyretic effect or the area would have to be connected to vasopressin-containing structures. Such is the case, in that a dense fibre network immunoreactive for AVP exists in the septal

area (Buijs, 1978; Buijs et al., 1978; Sofroniew, 1983). More specifically, a large amount of AVP immunoreactivity in densely packed terminals and thin fibres of passage within the VSA has been shown (DeVries et al., 1985). As well, large AVP-containing neurons project to the septal area (Conrad and Pfaff, 1975; Swanson, 1977; DeVries and Buijs, 1983). Thirdly, it has recently been shown that the septal area sends projections to vasopressin and oxytocin dendrites which are adjacent to the paraventricular nucleus (PVN) and the supraoptic nucleus (SON) and to vasopressin dendrites dorsal to the SCN or suprachiasmatic nucleus (Oldfield et al., 1985).

Electrophysiological data revealing afferent connections from the bed nucleus of the stria terminalis (BST) to the VSA indicated a possible source of antipyretic AVP (Disturnal et al., 1985). Immunohistochemical staining techniques have confirmed the BST to be the major source of vasopressin to the VSA (DeVries et al., 1985). It has also been suggested that AVP may be involved in thermoregulatory functions of the VSA since 75% of thermosensitive neurons in the VSA which responded to peripheral thermal stimulation also received afferents from the PVN and the BST (Disturnal et al., 1986).

A strong case for AVP modulation of fever was made when it was shown that the amount of immunoreactive AVP within the neuronal structures of the septal-hypothalamic system changed during that period of time when pregnant ewes were unable to produce fevers (Merker et al., 1980). Changes in the concentration of immunoreactive AVP within

various brain regions in response to endotoxin administration have also been demonstrated (Kasting and Martin, 1983).

For AVP to be a neuromodulator there must also be receptors in the septal area specific to AVP. Binding studies using labelled AVP have revealed a pattern of AVP binding in the septal area highly consistent with the distribution of immunostainable AVP fibres and the foci sensitive to local application of AVP (Baskin et al., 1983; Van Leeuwen and Wolters, 1983). Effective AVP antipyresis occurs only in this locus of the brain. Sites 2-3 mm dorsal, rostral or caudal are not responsive to AVP-induced antipyresis (Cooper et al., 1979; Ruwe et al., 1985a; Naylor et al., 1985a).

Mode of Action

By 1982 AVP was being considered as a homeostatic effector in the febrile process although its mode of action had not been determined (Kasting et al., 1982b). This suggestion has met with agreement from others who have suggested that AVP is active as a hormone, as a neurotransmitter (Pittman, 1981; Blatteis, 1981) and in fragments (Kovacs and deWeid, 1983). Others have suggested that the effects of AVP are receptor-mediated (Meisenberg and Simmons, 1984) and that the peptide neurotransmitter is released only at the synapse (Buijs and Heerikhuize, 1982). It has been shown that whereas AVP mediates its action in the kidney through cAMP, extrahypothalamic AVP does not mediate its action in the brain via the second messenger cAMP (Hawthorn and Jenkins, 1985). Although the mechanism of action is not yet understood it has been postulated that antipyresis results from the

acivation of neuronal pathways which release AVP from nerve terminals in the VSA during fever (Naylor et al., 1985).

Several suggestions as to how AVP might bring about the negative modulation of fever have been put forth.

1. AVP disrupts, directly, the events mediated by prostaglandins (Ruwe et al., 1985a)
2. AVP affects another component of the thermogregulatory system which then influences the processes to which the PGs are integral (Ruwe et al., 1985a)
3. AVP acts on other neuroactive substances to bring about its antipyretic effect, for example, AVP has corticotropin releasing activity and corticosteroids are anti-pyretic as well as anti-inflammatory (Kasting et al., 1982b).

As yet it is difficult to say what the exact mechanism of AVP action is in the modulation of fever. AVP is much more than an antipyretic however; it has a number of physiological effects which would be beneficial during infection and fever, such as antidiuretic effects, pressor effects, hormone release, enhancement of RES activity, or enhancement of memory processes, all of which would help to restore the organism to homeostasis (Kasting et al., 1982b).

Pharmacological Characteristics

It has been established that AVP is an antipyretic in the classical sense which Barbour defined early in this century. That is, AVP does

not lower normal body temperature when perfused within brain loci in which it is antipyretic (Kasting et al., 1978a, 1978b; Cooper et al., 1979; Naylor et al., 1985a). In contrast, AVP is hypothermic when administered:

1. Within the ventricles of the brain of humans (Cushing, 1931); rats (Kasting et al., 1980b); gerbils (Lee and Lomax, 1982)
2. Subcutaneously in the rat (Crine et al., 1981)
3. By direct microinjection (in relatively high doses) into the lateral septum, dorsal to antipyretic sites (Banet and Weiland, 1985).

The route and method of AVP administration as well as the discrete locus of administration all determine the nature of the central AVP effect. Small quantities and low doses of AVP which are push-pull perfused within a very discrete region of the forebrain result in dose-dependent decreases in febrile temperatures (Cooper et al., 1979). Perfusion of other neuroactive substances, for example oxytocin, did not elicit antipyretic effects (Lederis et al., 1982).

Antiserum specific to AVP interferes with the molecule itself and as a result blocks the antipyretic effect of AVP (Kasting, 1980; Malkinson et al., 1986). Furthermore, injected within the septum, a potent V1 receptor antagonist for AVP (Kruszynski et al., 1980) augmented the febrile response to IL1 in a dose dependent manner (Naylor et al., 1986a) while a potent V2 receptor antagonist (Krusynksi et al., 1980) had no effect on the fever. Finally, ventricular injection of the AVP analogue, DDAVP, which is an agonist of the peripheral V2 receptor,

did not effect an IL1-induced fever (Naylor et al., 1986b). These investigations indicate that AVP not only suppresses fever due to IL1, as well as BP and PGE, but that the antipyretic action does not involve the V2 subtype of the peripheral vasopressin receptor. Rather, the antipyretic receptor may be similar to the V1 subtype of the peripheral vasopressin receptor.

Other Possible Antipyretics

There are two other endogenous substances which have been suggested as endogenous antipyretic substances, adrenocorticotropin and alpha-melanotropin (Lipton et al., 1981). However, they have been researched to a much lesser degree, and with conflicting results. Suffice it to say that other peptides or endogenous neuroactive substances could interact with vasopressin to bring about a return to homeostasis in the febrile organism.

G. Rationale for Research

As early as 1970 extrahypothalamic areas of the brain were implicated in the pathogenesis of fever (Rosendorff and Mooney, 1971). Veale and Cooper (1975) lesioned the AHPOA of rabbits and found that the rabbits could still respond to pyrogen with a fever. Monkeys can also develop fever from pyrogens in the presence of the AHPOA lesions (Lipton and Trzcinka, 1976). This has led to the search for other brain sites which are sensitive to pyretics and antipyretics.

The ventral septal area is one such site. This area was found to be sensitive to the pyretic effects of PGE_1 (Rudy et al., 1977). Furthermore, the septal region was found to be the locus of an endogenous antipyretic system activated during a number of physiological challenges, including fever (Ziesberger, 1985; Kasting et al., 1985). The endogenous antipyretic substance was AVP, and this was supported by the fact that AVP perfused through the VSA reduced febrile temperatures due to endotoxin (Naylor et al, 1985a) and PGE_1 (Ruwe et al., 1985a). In contrast, in 1983 it was not known what effect an exogenous antipyretic, such as an aspirin-like drug, might have within the VSA.

The literature indicates a vasopressin antipyretic system within the Ventral Septal Area of the brain. The effect of aspirin-like drugs (ALDs) on such a system would not only be interesting in and of itself but could be of importance in the clinical treatment of fevers. It would be particularly important in light of the fact that AVP exposure sensitizes neuronal elements for a short time, so that a second exposure to AVP results in severe motor disturbances (Kasting et al., 1980b;

Naylor et al., 1985b). Such an effect is undesireable and may have long term implications for the health of the subject. Fever itself evokes AVP release to a certain degree and if the aspirin-like drugs were to enhance AVP release, the possibilities for febrile convulsions might be enhanced.

Another question which has arisen from the investigation of febrile mechanisms is whether or not prostaglandins are indeed the primary mediators of fever. The observation that ALDs block PG synthesis and release strongly reinforced the mediatory role whereas later evidence cast a shadow of doubt on PGs as the sole and primary mediator. Investigation of the effects of ALDs, within the VSA, on PGE fevers would provide additional information related to endogenous antipyresis.

The specific scientific questions addressed in this thesis are:

1. Does the classical antipyretic, sodium salicylate exert antipyretic actions in the same area of the brain where vasopressin antipyresis and PGE pyrogenicity have been demonstrated? If, as expected, sodium salicylate was not able to block PGE-induced hyperthermia then other fevers induced by IL1 or endotoxin would be challenged. A comparison with vasopressin-induced antipyresis would be made. If sodium salicylate administered within the VSA were able to block PGE-induced hyperthermia, subsequent research would address the question of how such an action was occurring.

2. In using sodium salicylate in the rat, attention must be paid to the reports of salicylate-induced hypothermia in the cold and occasionally at room temperature (Satinoff, 1972; Polk and Lipton, 1975). This suggests that salicylate (ip) might exert its action on thermoeffectors and not on the temperature set-point. The application of salicylate to the VSA in the cold would be an important control experiment.

3. If salicylate antipyresis were not the result of hypothermia then was the salicylate-induced blockade of PGE hyperthermia a result of enhancement or potentiation of vasopressin actions within the VSA? To address this question substances such as AVP antiserum or AVP receptor antagonists could be utilized.

II. Methods

Experiments were conducted on male Sprague-Dawley rats (250 - 350 g). The rats were housed in groups of 2 - 4 per cage at an ambient temperature of 22.0 ± 1.0°C in a room with controlled lighting (12 hr on - 12 hr off). Food and water were available ad libitum.

A. Surgical Procedure

For surgery, each rat was anaesthetized with 65/75 mg/kg sodium pentobarbital injected into the peritoneal cavity. When the rat was fully anaesthetized, the head area was shaven and then swabbed with a solution of 70% ethanol (95% ethanol and distilled water). Implantation of cannula in the brain proceeded using the following procedures:

1. The rat was placed in a stereotaxic apparatus, with head held in place with ear bars inserted into the ear canal and upper jaw held still with the incisor bar.
2. An incision was made anterior to posterior above the sagittal suture of the head so that the skin and underlying fascia could be held back, allowing access to the calvaria.
3. The bregma and lambda were marked and the angle of the head was adjusted so that the sagittal suture was horizontal.
4. The stereotaxic coordinates of Paxinos and Watson (1982) used to position the bilateral guide cannulae over the VSA were: 0.2 mm anterior to the Bregma, 1.0 mm lateral to the midline and 3.0 mm below dura. A ventricular guide had been soldered previously to the bilateral guides in such a way that its final coordinates were: 1.3

mm posterior to the Bregma, 1.5 mm lateral to the midline and 3.0 mm below dura.

5. The cannula tips were lowered to touch the skull and their position was marked on the skull surface. The carrier was then moved out of the way.
6. A dentist drill was used to bore holes through the skull for the guide cannula ensemble as well as for three stainless steel screws. The screws were then set into the skull as anchors for the guide ensemble.
7. The stereotaxic carrier was returned to its set position and the guide cannulae were then lowered into the brain such that the tips of the bilateral cannulae rested 5.0 mm above the VSA and the tip of the single cannula rested 0.5 mm above a lateral cerebral ventricle.
8. A small piece of porous gelfoam was fitted around the cannula to fill in the drilled area. Cranioplast cement was then used to secure the guides to the screws and skull. Several coats of cement were allowed to dry before gently removing the guide carrier from the guide ensemble.
9. The incision was sutured, the animal removed from the stereotaxic apparatus and snugly fitting stainless steel stylettes were used to occlude the guide cannulae. The rat was kept in a separate cage, under a heat lamp until he gained full consciousness whereupon he was returned to his original cage and allowed 7 - 10 days to recover.

B. Experimental Procedure

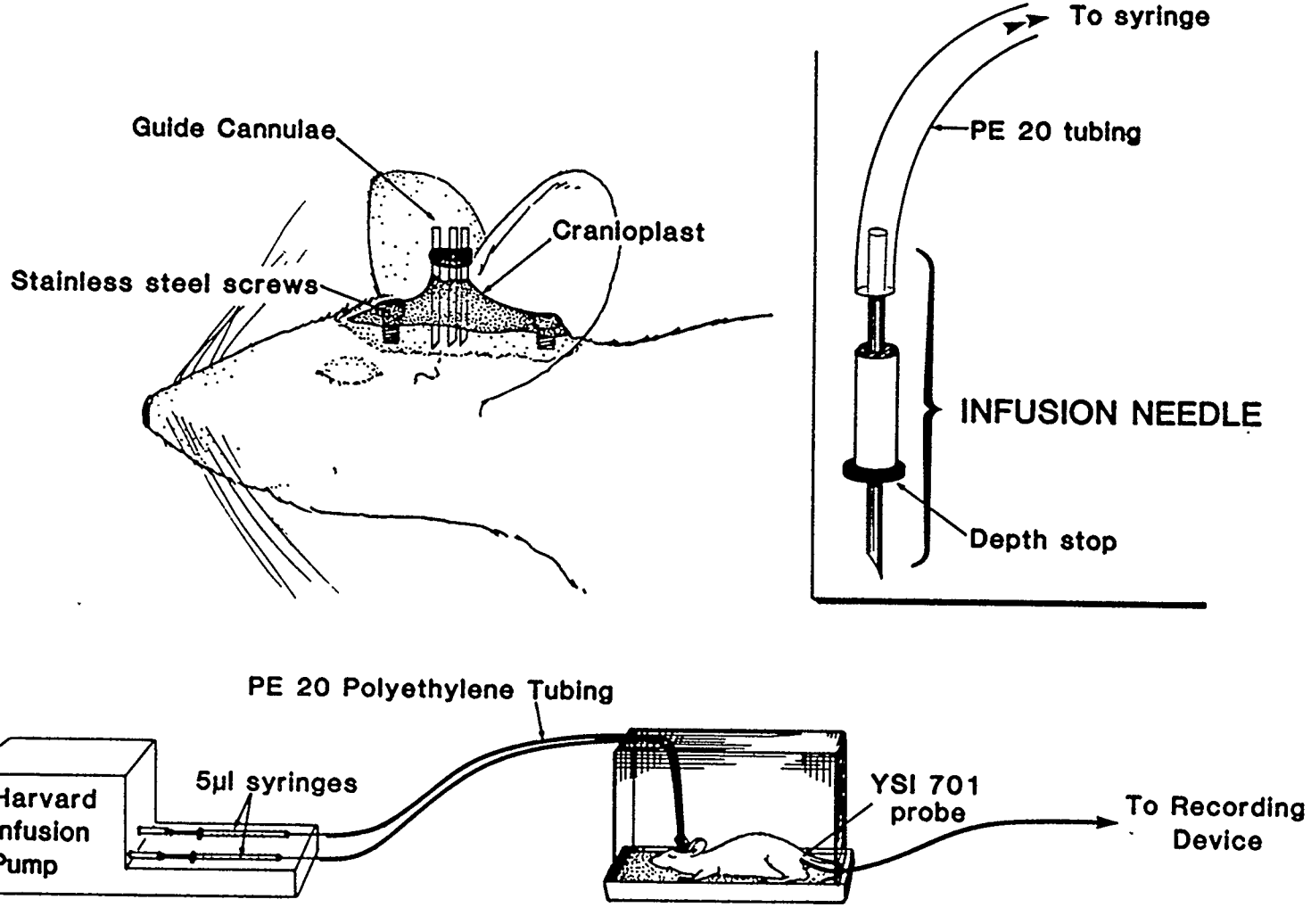
For experiments, rats had a YSI thermister probe inserted 6 cm beyond the anus and taped to the base of the tail. The rats were placed in a cage which had been modified to allow the rat to move in an unrestricted manner with the probe in place. Colonic temperature was monitored continuously on a datalogger (Digitech, 1101). The temperature of each rat was recorded in 5 - 15 minute intervals during the experiment, beginning at least one hour prior to infusion and continuing for 120 minutes following the termination of the infusion.

Artificial CSF (aCSF) and drug solutions (in aCSF) were infused bilaterally within the VSA through 27 gauge cannulae connected via polyethylene-20 tubing to 5.0 µl syringes mounted on an infusion pump (Harvard, 944). The pump had been calibrated previously to deliver 1.0 µl over a 1.0 hr period. Infusions were started 1.0 hr prior to and continued for 1.0 hr following the injection of PGE_1 (icv).

Microinjections of PGE_1 were made through a 27 gauge cannula which was connected to a length of polyethylene-20 tubing occluded at the opposite end with a stylet. The injection cannula was lowered through the guide tube to the level of the LCV. The PGE_1 (20 ng/µl) was injected in a volume of 10 µl over a period of 30 - 45 sec. A schematic representation of the experimental set-up is illustrated in Figure 3.

FIG. 3

A schematic representation of the methodological procedures employed for experiments. The upper left depicts positioning of the guide cannulae. The upper right panel illustrates the infusion needle which is inserted in the implanted cannulae. The lower portion of the figure illustrates the experimental set-up.


Guide Cannulae
Cranioplast
Stainless steel screws
To syringe
PE 20 tubing
INFUSION NEEDLE
Depth stop
PE 20 Polyethylene Tubing
Harvard
Infusion
Pump
5µl syringes
YSI 701
probe
To Recording
Device

Upon completion of an experimental series, each rat was anaesthetized deeply with sodium pentobarbital and then the brain was perfused with saline followed by neutral formalin (4% formaldehyde, 0.9% saline, deionized water). Sections were then stained with neutral redsolution and injection sites were localized by light microscopy (refer to Section D).

Data were subjected to a one-way analysis of variance followed by the Scheffe post-hoc test (Hinkle et al., 1979). The 1.0 hr fever indicies (area under each fever curve for a 1.0 hr period following the injection of PGE1) were subjected to the Student's t-distribution for paired or unpaired samples. Results were considered to be significant at $p < 0.05$.

C. Solutions

Artificial CSF was mixed according to the following formula:

Solution A

NaCl	15.194 g
KCl	0.448 g
NaH_2PO_4	0.300 g
$CaCl_2.2H_2O$	0.352 g
$MgSO_4.2H_2O$	0.592 g

mixed in 1.0 L of distilled H_2O

Solution B

$NaHCO_3$	4.032 g
Mannitol	3.604 g

mixed in 1.0 L of distilled water

The pH was adjusted to 7.4 with approximately 4 drops of HCl solution. Equal portions of these solutions A and B were mixed together before experiments. The aCSF solution was passed through a 0.22 µm filter unit (Millipore, Millex-GV) as an extra precaution against pyrogen contamination.

Sodium Salicylate (Fisher Scientific) was prepared in sterile aCSF solution in concentrations of 10.0, 30.0, 50.0 and 100.0 µg per µl of aCSF. The pH was adjusted to 7.4 ± 0.2 by adding HCl solution. Salicylate solutions were also passed through a 0.22 µm filter unit. All solutions were stored at 4.0°C.

A stock solution of PGE1 was prepared with 2.0 mg of prostaglandin E1 (Upjohn) dissolved in 1.0 ml of 95% ethanol. For experiments, 10.0 µls of stock solution were added to 1.0 ml of aCSF rendering a concentration of 20.0 ng/µl. The PGE1 was given by gravity flow into the ventricle in a volume of 10.0 µls. After one minute the injection cannula was removed and the stylette was returned to the guide cannula.

A highly specific antiserum for AVP, prepared according to the method of Moore et al., (1977), was kindly provided by K. Lederis. For experiments the antiserum was diluted in a 0.01 M phosphate buffer solution containing: $NaPO_4$, KPO_4, NaCl, NaAzide, bovine serum albumin and physiological saline.

To antagonize endogenous AVP effects, a specific and potent antivasopressor known as d(CH_2)5 Tyr (Me) AVP was obtained from M. Manning of The Medical College of Ohio by way of Q. Pittman of the University of Calgary. This antagonist has been shown to be very effectiv in blocking the central effects of arginine vasopressin (Kruszynski et al., 1980; Naylor et al., 1986d; Albers et al., 1986).

D. Histology

Kill-perfusion Procedure

Upon completion of an experimental series, each rat was anaesthetized deeply with sodium pentobarbital (i.p.), an 18 gauge needle was inserted into the left ventricle of the heart and held in place with a hemostat. The right atrium of the heart was punctured and saline was then perfused through the needle and followed by neutral formalin. This method perfused and fixed the brain tissue.

The brain was removed and stored in neutral formalin for 3 - 10 days, transferred to sucrose acacia for 1 - 2 days and then blocked on the coronal plane and sectioned at 40 µm on a sledge microtome. Sections were mounted on slides coated with potassium dichromate and gelatin and dried at room temperature for several days or in a drying oven at 30°C for 24 hours.

Staining Procedure

The sections were then stained in the following manner:

1. 4.5 minutes in Neutral Red solution bath (0.5 g Neutral Red C.I. 50040 in 100.0 mls 50% ethanol)
2. 2.0 minutes in distilled water bath
3. 3.0 minutes in second distilled water bath
4. 2.0 minutes in 70% ethanol (to differentiate)
5. 3.0 minutes in 95% ethanol (to dehydrate)
6. 3.0 minutes in xylene bath (to clear and dehydrate)
7. 1.0 - 3.0 minutes in second xylene bath

The slides were removed from the xylene one at a time and immediately coated with histological mounting medium (Permount, Fisher Scientific) and a cover slip.

The precise location of the infusion site was visualized under a light microscope using the rat brain atlas of Paxinos and Watson (1982) for anatomical reference. Infusion sites which were not within the ventral septal area were noted and the corresponding data was used for other site control groups or not used for statistical analysis.

E. Data Analysis

Rectal temperatures at the time of PGE1 injection (Hour 0) were designated as baseline temperature and subjected to an analysis of variance to establish that they were not significantly different between groups. Then rectal temperatures were converted to deviations from the baseline temperature. These data were then subjected to an one-way

analysis of variance followed by the Scheffe post-hoc test. The analysis of variance could compare the data for four groups at one time. The Scheffe post-hoc test is a conservative test and is particularly recommended when the group sample sizes are unequal or when complex comparisons are made (Hinkle et al., 1979). Such a test was required through the course of this research.

The fever index has been included in the analysis and presentation of the data because it provides a quantitative measure of the febrile response. The index is the area contained under the fever curve for a designated period of time. In this thesis it is presented as °C.hr for the one hour period following exposure to prostaglandin. The index values were subjected to the Student's t-distribution for unpaired samples.

Results were considered to be significant if $p < 0.05$ and are presented as means plus or minus the standard error of the mean (mean ± S.E.M.).

III. SODIUM SALICYLATE WITHIN THE VENTRAL SEPTAL AREA: EFFECT ON PGE_1-INDUCED HYPERTHERMIA

A. Introduction

One question which has arisen from the investigation of febrile mechanisms is whether or not prostaglandins of the E series are primary mediators of fever (Milton and Wendlandt, 1971; Feldberg and Gupta, 1973; Coceani, 1974). The more important pieces of evidence for a primary role include:

1. PGE concentration in the CSF increases during pyrogen-induced fever (Feldberg and Gupta, 1973; Coceani et al., 1983; Cranston et al., 1984)
2. Aspirin-like drugs block PGE synthesis and release (Vane, 1971; Flower and Vane, 1972)
3. PGE causes a marked increase in body temperature when administered directly into the cerebral ventricles (icv), the AHPOA and the VSA (Feldberg and Saxena, 1971; Veale and Cooper, 1972; Williams et al., 1977)

There are numerous other observations which have been discussed in the Introductory section of this thesis which are not repeated here.

Alternatively, a number of observations have been made which do not support a mediatory role for prostaglandins. For example:

1. The PGE concentration in CSF has been reduced with aspirin-like drugs at the same time that a febrile temperature was being maintained (Cranston et al., 1975a)
2. PGE antagonists given icv can inhibit fever induced by icv PGE injection but not by icv leucocytic pyrogen injections (Cranston et al., 1976)
3. Although EP could elicit a fever in AHPOA-lesioned rabbits, intrahypothalamic prostaglandin E1 could not increase core temperature (Veale and Cooper, 1975)

Although their role as the primary mediator of fever has been called into question, the ability of prostaglandins to cause fever when administered into sensitive brain sites is well established.

The role of PGE in fever has been supported strongly by the observation that aspirin-like drugs inhibit the synthesis and release of prostaglandin (Vane, 1971). For instance, it is well known that sodium salicylate suppresses pyrogen-induced fever when administered within central areas such as the AHPOA and midbrain (Cranston and Rawlins, 1972; Avery and Penn, 1974; Schoener and Wang, 1975; Vaughn et al., 1979). Additional evidence from electrophysiologicl studies on AHPOA neurons has shown that sodium salicylate reverses the inhibitory effects of pyrogen on warm-sensitive neurons and the excitatory effects on cold-sensitive neurons (Schoener and Wang, 1975a; Hori et al., 1984). These observations support the idea that aspirin-like drugs are antipyretic via their inhibition of prostaglandin synthesis from pyrogen-activated processes.

Peripherally administred aspirin-like drugs do suppress pyrogen-induced fevers, but do not block PGE-induced hyperthermia (Milton and Wendlandt, 1971; Schoener and Wang, 1974; Woolf et al., 1975; Clark and Cumby, 1975). The effect of intracranially administered aspirin-like drugs on PGE-induced thermogenesis in conscious animals has not been reported. This is not surprising since the mode of action of aspirin-like drugs is thought to occur via the inhibition of prostaglandin synthesis and release. That is, the effects of the exogenous, ventricularly administered PGE presumably would not be affected by the action of exogenous, centrally administered aspirin-like drugs. However, PGE-induced hyperthermia has been suppressed by the endogenous antipyretic peptide, arginine vasopressin, administered within the ventral septal area of the rat (Ruwe et al., 1985). Furthermore, the VSA has been suggested recently as the site where AVP might have a role to play in the modulation of fever (Veale et al., 1984). The experiments described here were set out to determine if the VSA, where both prostaglandin and vasopressin are able to produce and reduce hyperthermia respectively, might be sensitive to the antipyretic effects of the aspirin-like drugs.

B. Results

Sodium salicylate (30 µg/µl) infused into the VSA for one hour prior to and one hour following an intraventricular dose of PGE_1 caused a significant suppression of the hyperthermic response which was seen during the control infusion. Figure 4 illustrates the mean core temperature responses during control and sodium salicylate infusions (n = 12). Rats receiving aCSF in the VSA responded to PGE_1 in the

ventricle with a temperature increase of 0.92°C ± 0.15°C at 30 minutes post-injection. However, during the infusion of salicylate, the PGE_1 hyperthermia was reduced significantly to 0.32°C ± 0.15°C at 30 minutes ($p < 0.01$). Temperatures during salicylate infusion were also significantly different 15 minutes after PGE injection and at all other times intervals measured. The infusion of either aCSF alone or with sodium salicylate did not have a significant effect on body temperature prior to PGE_1 injection.

Figure 5 depicts fever indices for the same group of 12 animals. The fever index indicates the area contained under the fever curve for a period of one hour following the injection of PGE_1 into the ventricle and is a quantitative measure of the febrile response. The animals receiving sodium salicylate infusions had significantly smaller areas under the fever curves than those of animals receiving aCSF infusions ($p < 0.01$).

Histological examination of the 12 brains indicated that the sites of infusions were located in the ventral septal area. A schematic representation of a coronal section of the rat brain at 0.2 mm anterior to the bregma, according to the atlas of Paxinos and Watson (1982), is shown in Figure 6. The areas of tissue into which the bilateral microinfusions of sodium salicylate reduced the PGE-induced hyperthermia are indicated.

FIG. 4

Mean temperature responses (± S.E.M.) in 12 animals. A 2.0 h infusion of aCSF alone did not affect the PGE_1-induced hyperthermia. Infusion of 30.0 µg/µl sodium salicylate into the VSA significantly suppressed the hyperthermic response to icv PGE_1 (* $p < 0.05$; ** $p < 0.025$; *** $p < 0.01$; t $p < 0.005$; tt $p < 0.001$).

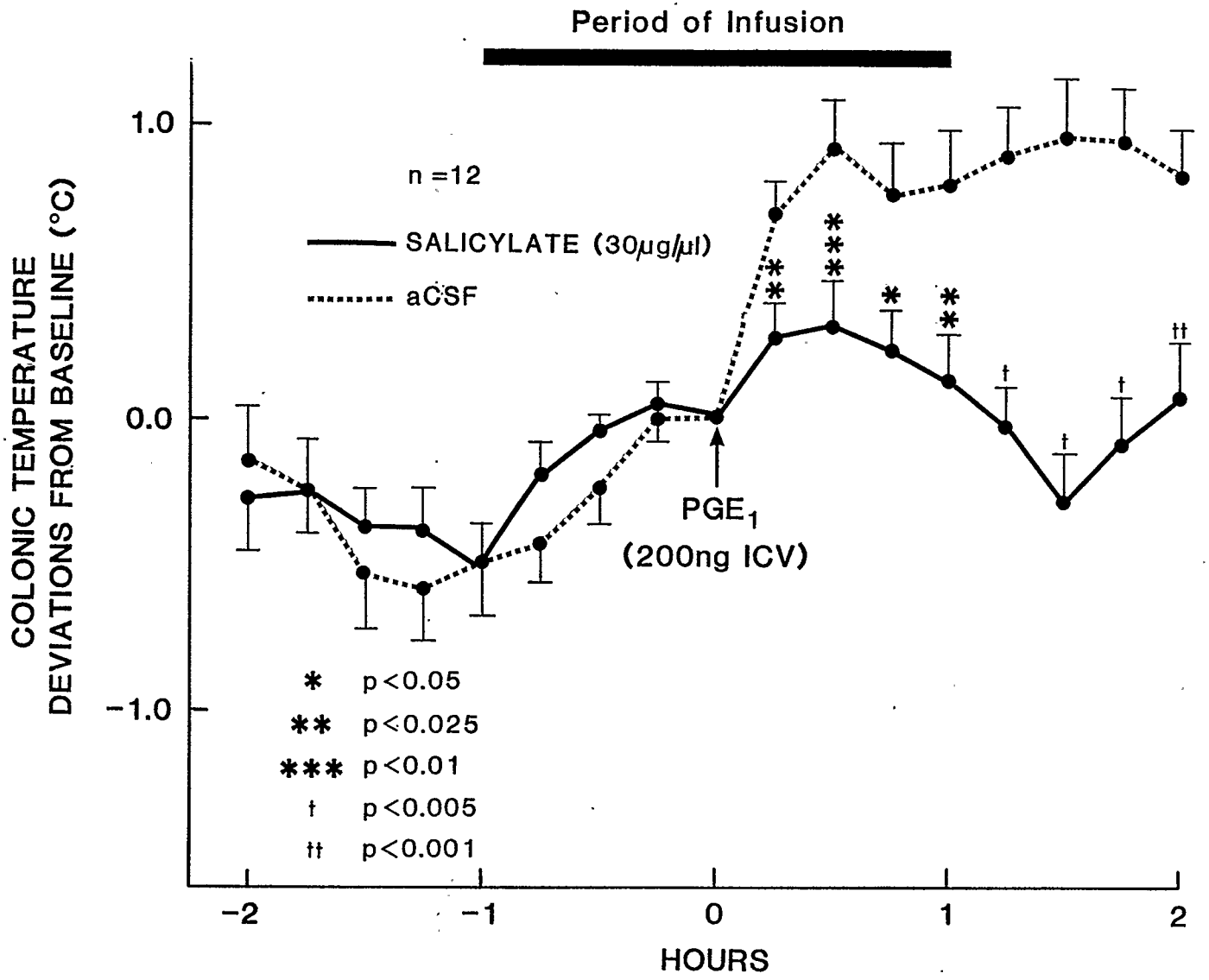

Period of Infusion
n =12
SALICYLATE (30μg/μl)
aCSF
PGE1
(200ng ICV)
* p<0.05
** p<0.025
*** p<0.01
† p<0.005
†† p<0.001
COLONIC TEMPERATURE
DEVIATIONS FROM BASELINE (°C)
1.0
0.0
-1.0
-2
-1
0
1
2
HOURS

FIG. 5

The 1.0 h fever index (mean ± S.E.M.) following PGE_1 injection (icv) was significantly reduced ($p < 0.01$) during infusion of sodium salicylate as compared to aCSF alone (aCSF = 0.69 ± 0.13 °C.h; salicylate = 0.22 ± 0.10 °C.h).

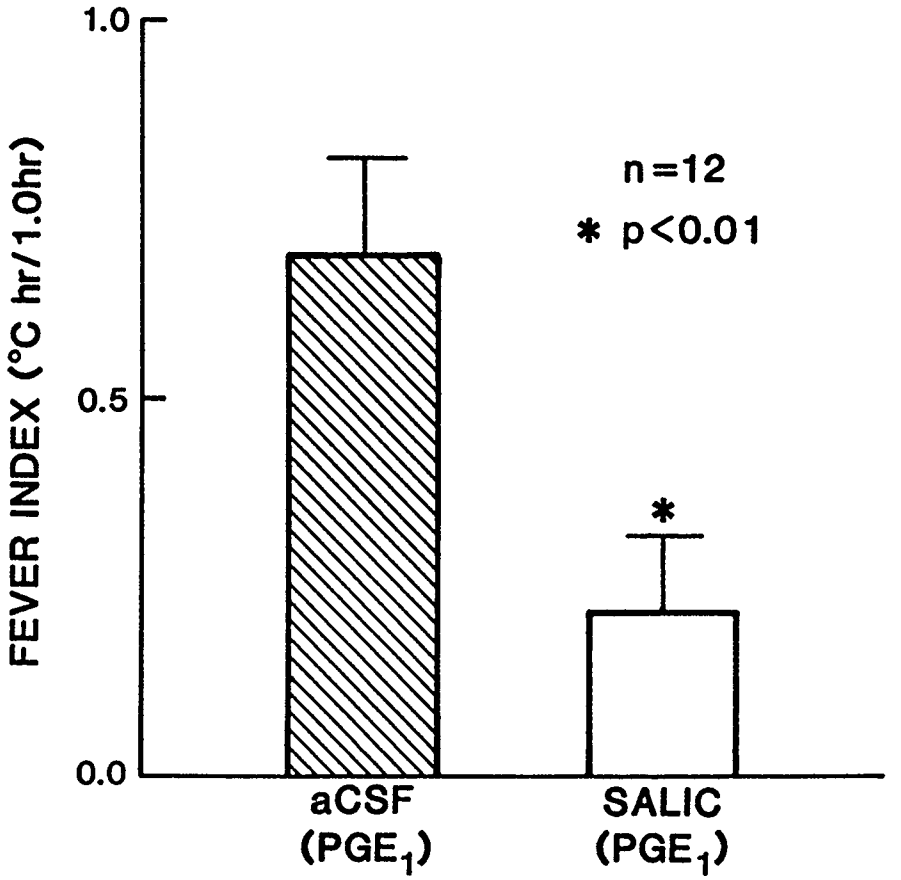
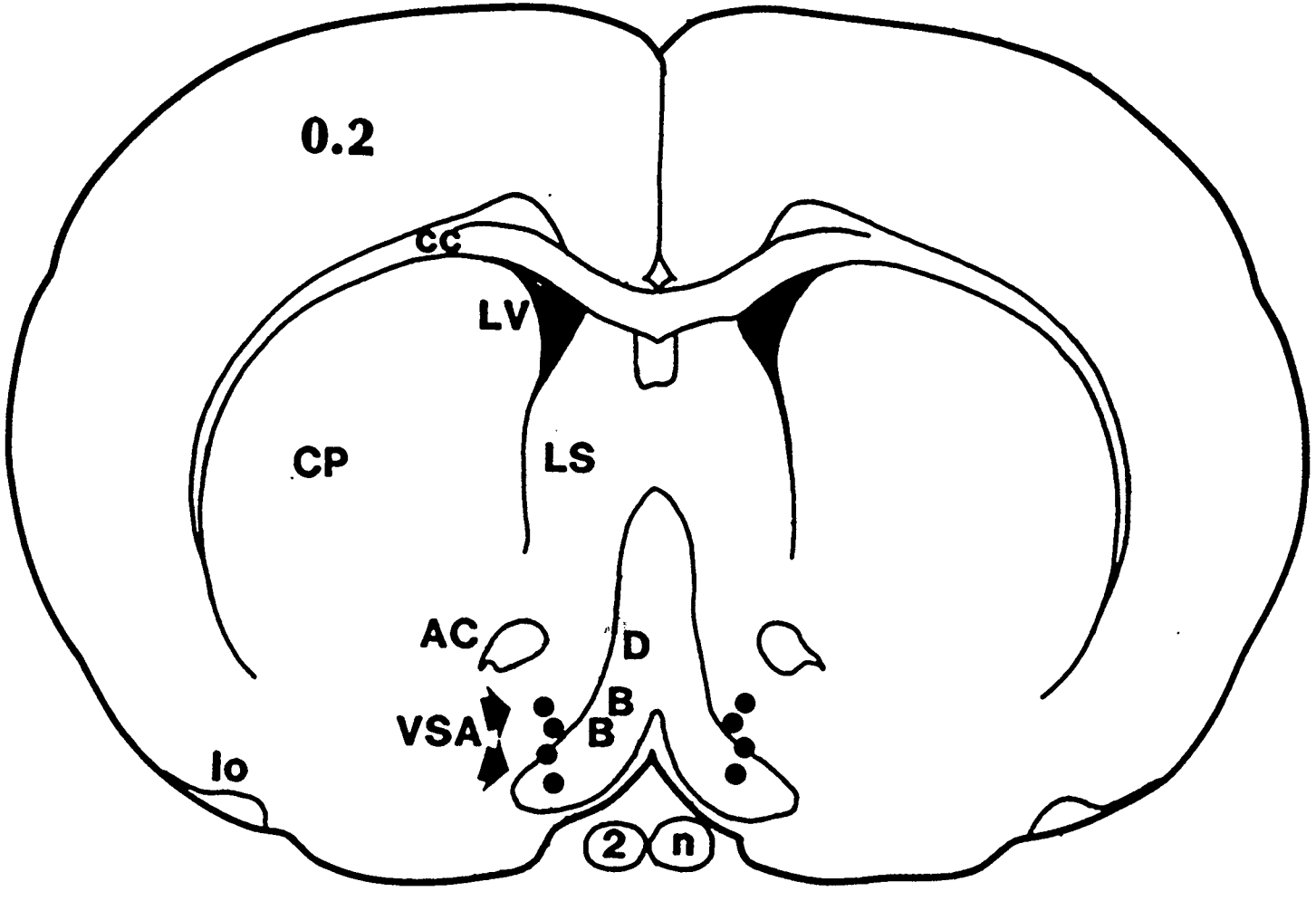

1.0
0.5
0.0
FEVER INDEX (°C hr/1.0hr)
n=12
* p<0.01
*
aCSF
(PGE1)
SALIC
(PGE1)

FIG 6

Schematic representation of a coronal section of the rat brain at 0.2 mm anterior to the Bregma. The filled circles indicate the areas of tissue into which all bilateral infusions of aCSF alone were ineffective but where all infusions of sodium salicylate were effective in reducing hyperthermia. These areas correspond to the ventral septal area of the rat brain. Abbreviations: AC, anterior commissure; cc, corpus callosum; CP, caudate putamen; DBB, diagonal band of Broca; lo, lateral olfactory tract; LS, lateral septal nucleus; LV, lateral cerebral ventricle; 2n, optic nerve; VSA, ventral septal area.


0.2
cc
LV
CP
LS
AC
D
VSA
B
B
lo
2
n

Tracts left by the infusion process can be seen in Figure 7 which is a photograph of a brain slice 0.2 mm anterior to the Bregma. The tracts reach down to the level of the VSA. The lack of necrosis beyond the region of the cannulae was observed in the majority of the brain slices. Yaksh and Yamamura (1974) found that when isotonic solutions were perfused, tissue damage did not occur much beyond the cannula tip. Another way to determine damage to tissue has been to measure concentrations of intracellular enzymes in the perfusate and after initial trauma from cannula insertion, the levels of such enzymes were found to remain low (Honchar et al., 1979). Rosendorff (1969) has shown that repeated injections of 10 µl of fluid into the AHPOA had no effect on rectal temperature. In further support of the minimal impact the infusions in these experiments actually had, Myers (1971) has demonstrated that the volume of injected substance determines the spread of injectate through tissue. Furthermore, the spread of injectate can be limited by employing a slow infusion rate and small injection volumes which also help to minimize tissue destruction at the tip of the cannulae (Myers, 1971). Perhaps then, the low amount of tissue destruction observed in the brain slices from these experiments is due to the slow infusion of a small volume of solution (1.0 µl/h).

A comparison of site sensitivity indicated that infusions of sodium salicylate (30 µg/µl) at a level dorsal to the VSA did not produce the same suppression of PGE-evoked hyperthermia. Figure 8 shows the record of the mean temperature response of 6 animals to PGE_1 during infusions of sodium salicylate and aCSF at sites dorsal to the VSA, near the

FIG. 7

Photograph of a brain slice at 0.2 mm anterior to the Bregma and stained with neutral red solution. The lesions left by the infusion needles indicate that the level of the ventral septal area was reached during experiments (arrowheads).

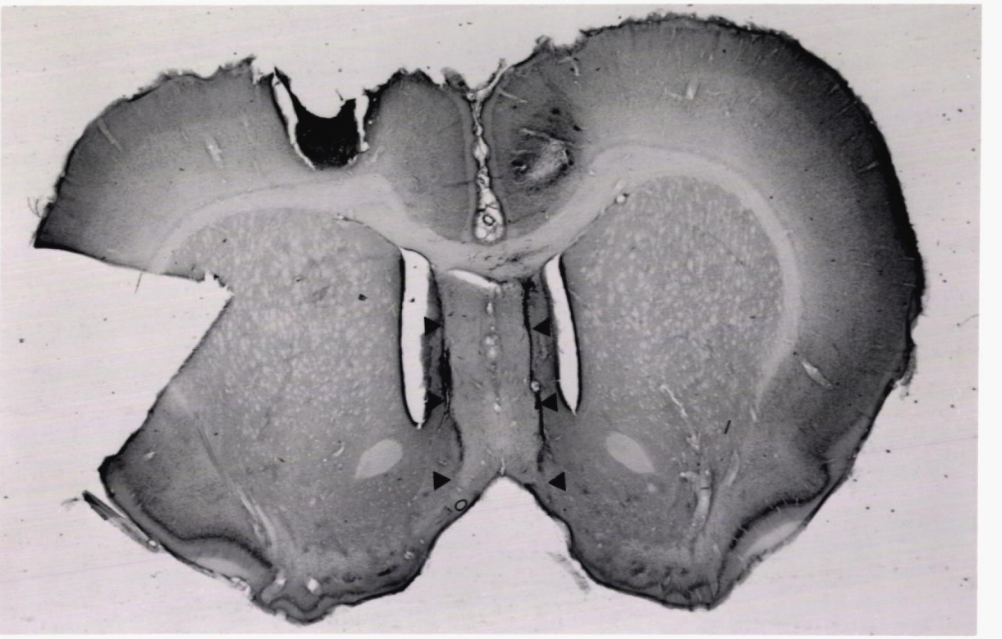


FIG. 8

Mean colonic temperature (± S.E.M.) in 6 animals. Bilateral infusions of aCSF alone or with sodium salicylate (30.0 µg/µl) at a level dorsal to the VSA were without effect on PGE_1-induced hyperthermia. The inset is a schematic representation of a coronal section of the rat brain at 0.2 mm anterior to the Bregma. The filled stars indicate the area of tissue into which all infusions were made without effect on hyperthermia. This area is dorsal to the VSA, in the vicinity of the anterior commissure of the rat brain. Abbreviations are as in FIG. 6.

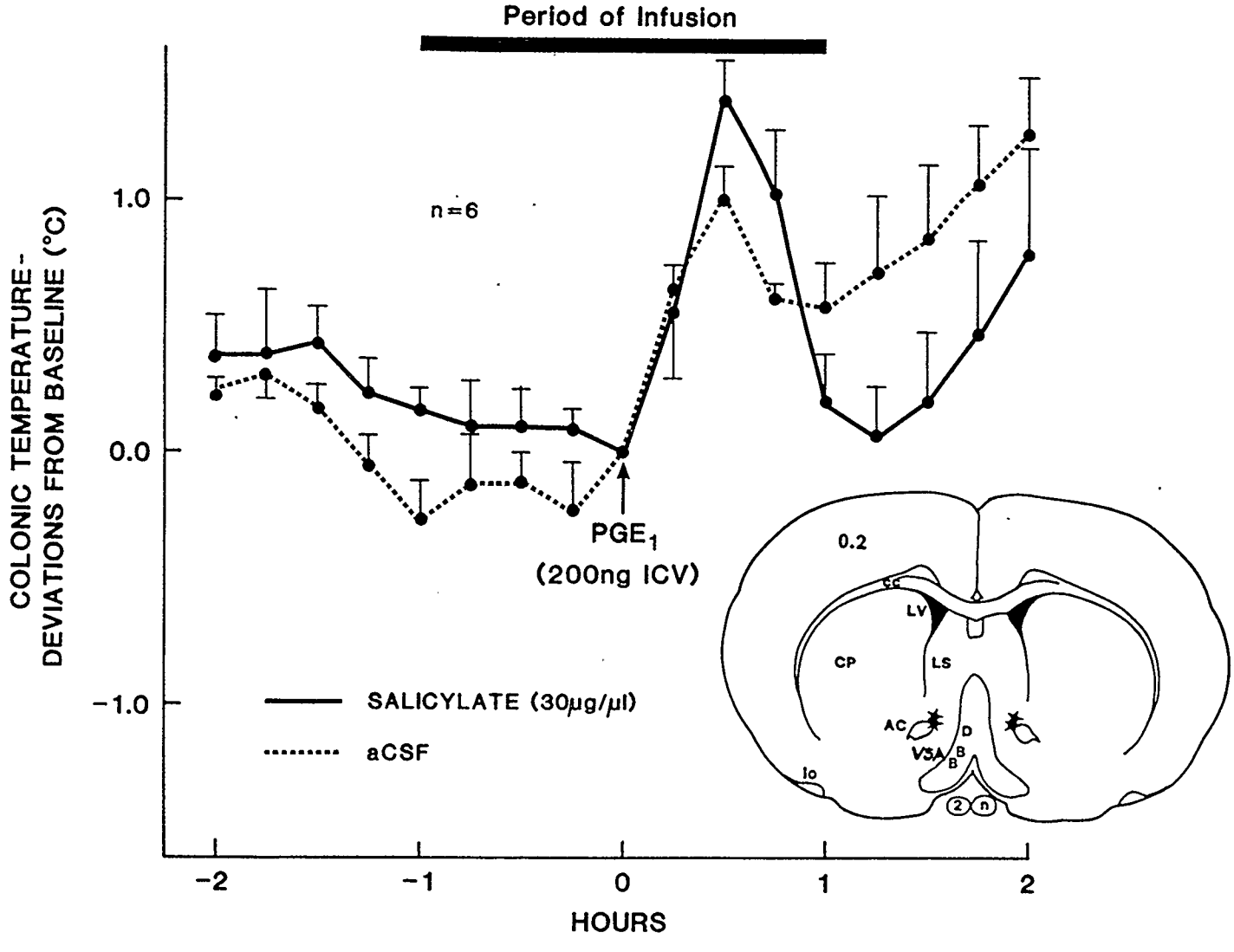
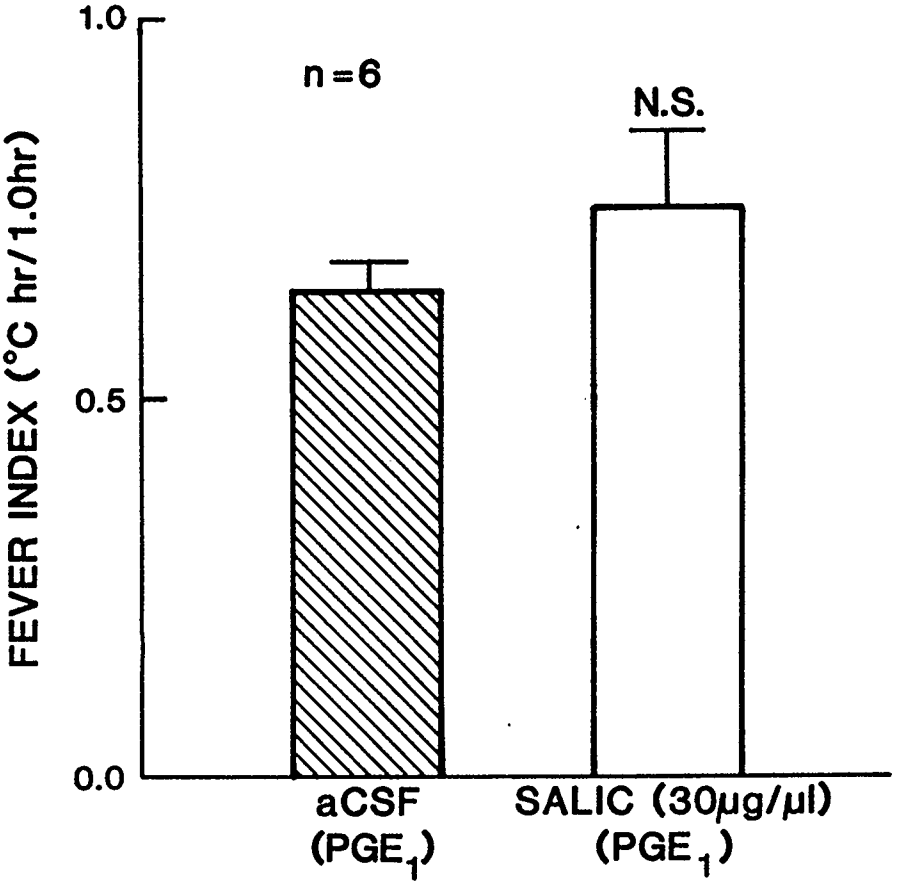

Period of Infusion
n=6
COLONIC TEMPERATURE-
DEVIATIONS FROM BASELINE (°C)
1.0
0.0
-1.0
PGE1
(200ng ICV)
SALICYLATE (30µg/µl)
aCSF
-2
-1
0
1
2
HOURS
0.2
LV
CP
LS
AC
D
V3A
B
Io

FIG. 9

The 1.0 h fever index (mean ± S.E.M.) following PGE_1 injection (icv) was not significantly different during infusion of sodium salicylate (30.0 µg/µl) into the area of the anterior commissure as compared to aCSF alone (aCSF = 0.64 ± 0.04 °C.h; salicylate = 0.75 ± 0.10 °C.h).


1.0
0.5
0.0
FEVER INDEX (°C hr/1.0hr)
n=6
N.S.
aCSF
(PGE1)
SALIC (30µg/µl)
(PGE1)

anterior commissure. There was no significant difference between the two treatments before, during or after the period of infusion. The inset diagram shows brain sites at which sodium salicylate was ineffective in reducing PGE_1 hyperthermia. The fever indicies, shown in Figure 9, indicate no quantitative difference between salicylate and aCSF exposure at the level of the anterior commissure. The mean values (± S.E.M.) for sodium salicylate and aCSF infusions in the two regions Of the brain are summarized in Table 1.

	FEVER INDEX (°C.h/1h)		RECTAL TEMPERATURE (°C) @ 15 mins		@ 30 mins	
	VSA	AC	VSA	AC	VSA	AC
SODIUM SALICYLATE (30 µg/ul)	0.22 ± 0.10	0.75 ± 0.10	0.28 ± 0.12	0.55 ± 0.24	0.32 ± 0.15	1.42 ± 0.15
artificial CSF	0.69 ± 0.13	0.64 ± 0.04	0.69 ± 0.12	0.63 ± 0.13	0.92 ± 0.15	1.02 ± 0.12

Table 1: Summary of mean experimental values (± S.E.M.) for 12 animals infused within the ventral septal area (VSA) and 6 animals infused near the anterior commissure (AC) with aCSF or sodium salicylate (30.0 ug/ul) for 1.0 hour prior to and 1.0 hour following PGE_1 (icv).

C. Discussion

The results of these experiments demonstrated that a bilateral infusion of 30.0 µg/µl/hr of sodium salicylate within the VSA suppressed, by a maximum of 62%, the hyperthermia due to PGE_1 injected into a lateral ventricle. The attenuation of PGE-induced hyperthermia began immediately and lasted the course of the experiment. Previous experiments had shown that 30.0 µg/µl of sodium salicylate within the AHPOA was an appropriate dose to be used in the rat (Cranston and Rawlins, 1972; Vaughn et al., 1979).

The present experiments would seem to indicate a mechanism of central action for sodium salicylate other than the inhibition of prostaglandin synthesis and release established by Vane (1971). Previous experiments have tested the effect of centrally administered salicylate on leukocyte progen applied centrally (Schoener and Wang, 1975; Hori et al., 1984) or peripherally (Cranston and Rawlins, 1972; Avery and Penn, 1974). In these cases the salicylate-induced antipyresis had concurred with the mechanism of action of aspirin-like drugs mentioned above. No reports were available in the literature of experiments which had tested the central (or specifically septal) application of sodium salicylate against a centrally-induced prostaglandin hyperthermia. If aspirin-like drugs reduce fever in the brain solely by their inhibition of prostaglandin synthesis and release then the present results should not have occurred. The manner in which sodium salicylate within the VSA was inducing antipyretic effect was not associated with its effect on the cyclo-oxygenase enzyme, unless PGE given icv promotes further PGE synthesis in the brain substance, or

contains pyrogens as contaminants. However reports in the literature up to date make these latter possibilities unlikely.

The ability of sodium salicylate to block PGE hyperthermia may be explained by the site-specificity demonstrated in these experiments. The area of infusion was contained within the ventral regions of the septum bordering on the diagonal bands of Broca. Infusions occurring dorsal to the VSA, near the anterior commissure, did not elicit the same response. Infusions occurring more caudally, toward the anterior hypothalamus, also did not elicit the antipyretic response. The results herein are specific to the VSA. There are several characteristics of the VSA which suggest that it is a unique site for a role in the mediation of fever. Firstly, the endogenous peptide, arginine vasopressin, suppressed similar PGE-induced hyperthermia when perfused in the VSA (Ruwe et al., 1985). Secondly, PGE infused within the VSA induced a marked hyperthermic response (Williams et al., 1977). Thirdly, AVP-containing neurons have been localized in this region (Conrad and Pfaff, 1975; Buijs, 1978; Buijs et al., 1978). Fourthly, connectivity from the PVN, BST and SCN (major sources of AVP) to thermoresponsive neurons in the VSA has been demonstrated (Disturnal et al., 1985, 1986). Thus it was postulated that the infusion of salicylate could enhance the release of endogenous arginine vasopressin or enhance the effects of AVP, thereby eliciting an antipyretic action against centrally-induced PGE hyperthermia.

IV. DOSE RESPONSIVENESS OF SODIUM SALICYLATE ANTIPYRESIS WITHIN THE VENTRAL SEPTAL AREA

A. Introduction

The ability of a 30.0 µg/µl dose of sodium salicylate to block the hyperthermic effects of exogenously administered PGE was further investigated by testing if the effect of sodium salicylate within the VSA was dose-related. Clinical work has shown that the effects of antipyretic drugs are dose responsive (Thiessen, 1982). Dose responsiveness can indicate that an effect is receptor mediated especially when the effect is investigated in the presence of antagonists to the drug being tested (Bowman and Rand, 1980). Indeed it has been suggested that antipyretics can directly antagonize the action of leukocytic pyrogen at the LP receptor sites in the CNS (Clark and Coldwell, 1972; Clark, 1980). Given these observations it seemed likely that sodium salicylate applied within the VSA blocked PGE-induced hyperthermia in a dose-related manner.

Reports in the literature were used to estimate a range of doses which have been antipyretic. Cranston and Rawlins (1972) found that a bolus injection (10.0 µls) of 6.0 - 30.0 µg of salicylate in the AHPOA of rabbits produced a reduction in fever due to iv pyrogen. Avery and Penn (1974) microinjected 5 µg doses (in 0.5 µls) into the AHPOA of rats and observed the reduction of endotoxin fever. Schoener and Wang (1975a) injected 100 µg doses of salicylate (in 0.5 - 1.0 µls) onto AHPOA thermosensitive neurons of cats and reversed the pyrogen-induced changes in neuronal firing rate. Using an infusion technique similar to

that of the present research, Vaughn et al. (1979) found that salicylate concentrations of 100.0 and 200.0 µg/µl/hr infused within the AHPOA of rabbits were effective against pyrogen-induced fevers while 50.0 µg/µl/hr was not effective.

Thus, a dose range of 5.0 - 200.0 µg of sodium salicylate had been tested within the hypothalamic tissue of laboratory animals. The concentration of salicylate administered within the brain leading to antipyresis varied from 0.6 - 200 µg/µl. Grundmann found that 300 mg/kg sodium salicylate given iv, which resulted in antipyresis, led to brain CSF concentrations of 25 ng/µl and hypothalamic salicylate concentrations of 1.57 - 1.76 mg/100 g of tissue. In contrast, 0.5 mg of salicylate given icv resulted in hypothalamic salicylate concentrations of 4.93 mg/100g of tissue which is three times greater than that produced by the intravenous dose. It therefore appeared that a 10 µg dose, at the lower end, and a 100 µg dose at the upper end of the dose range would be appropriate. A third intermediate dose of 50.0 µg was chosen as it was sufficiently close to the 30.0 µg dose used in the original experiments. It was postulated that if the antipyretic effect was dose-responsive, 10.0 µgs would attenuate PGE hyperthermia to a lesser degree than the 50.0 µg or 100.0 µg doses. As well, since the 30.0 µg dose reduced PGE hyperthermia by approximately 62%, it was postulated that the 50.0 and 100.0 µg doses would attenuate the hyperthermia to an even greater degree.

B. **Results**

Results of the dose test verified the original observation that sodium salicylate infused within the VSA was able to block PGE-induced hyperthermia. Infusions of artificial CSF (control) had no effect on the hyperthermic temperatures caused by an intracerebroventricular injection of PGE_1 (0.94 ± 0.14°C). During the infusion of 10.0 µg/µl of sodium salicylate, the increased body temperatures induced by PGE_1 were not significantly different (1.22 ± 0.30°C) from control temperatures. However, infusion of 50.0 µg/µl sodium salicylate within the VSA suppressed significantly (0.31 ± 0.19°C) the PGE_1-induced hyperthermia. Body temperatures during the infusion of 100.0 µg/µl doses of salicylate, although they tended to be lower than control temperatures, did not significantly differ from controls until 45 minutes following PGE_1 injection (0.68 ± 0.23°C). Figure 10 indicates the mean core temperature responses of rats during control and sodium salicylate infusions at the three doses (Panel A, 100.0 µg/µl; Panel B, 50.0 µg/µl; Panel C, 10.0 µg/µl).

Figure 11 depicts fever indices for the three different doses of sodium salicylate and of aCSF, as well as the original 30 µg/µl treatment. The responses of animals receiving sodium salicylate infusions of 50.0 and 100.0 µg/µl had significantly less area ($p < 0.005$ and $p < 0.05$ respectively) under the fever curves than those animals receiving either aCSF or 10.0 µg/µl sodium salicylate.

FIG. 10

Mean temperature responses (± S.E.M.) of 3 groups of animals. A 2.0 h infusion (indicated by the dark bar above the curves) of aCSF (solid line) or aCSF with sodium salicylate (broken line) at 3 different concentrations resulted in varying levels of antipyresis. Panel A: infusion of 100.0 µg/µl sodium salicylate did not differ significantly from aCSF until 45 minutes after the injection of PGE_1 (icv; indicated by the arrow at 0 h). Panel B: infusion of 50.0 µg/µl sodium salicylate significantly differed from aCSF infusion at all times following PGE_1. Panel C: infusion of 10.0 µg/µl sodium salicylate did not significantly differ until 45 minutes after PGE_1 (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

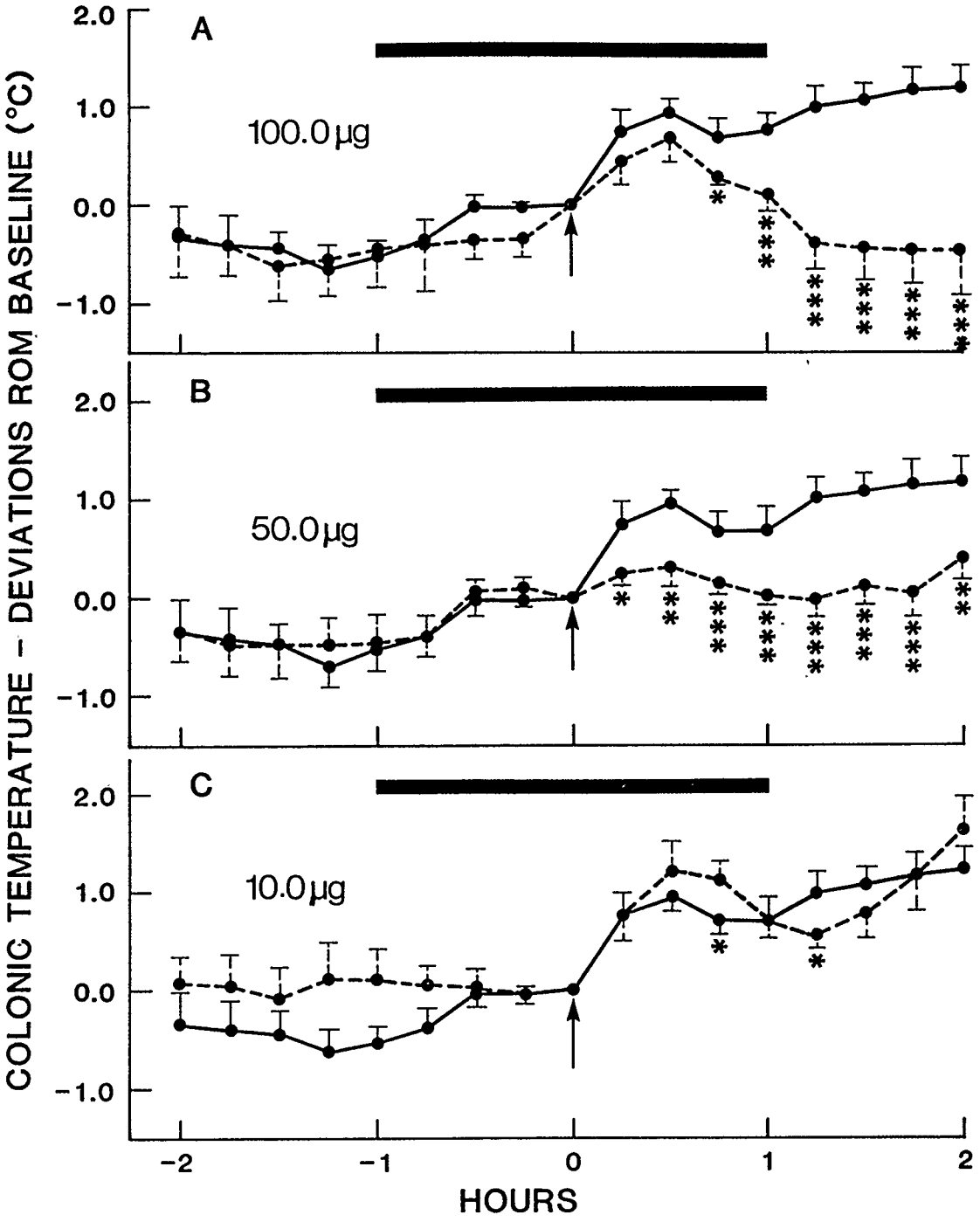
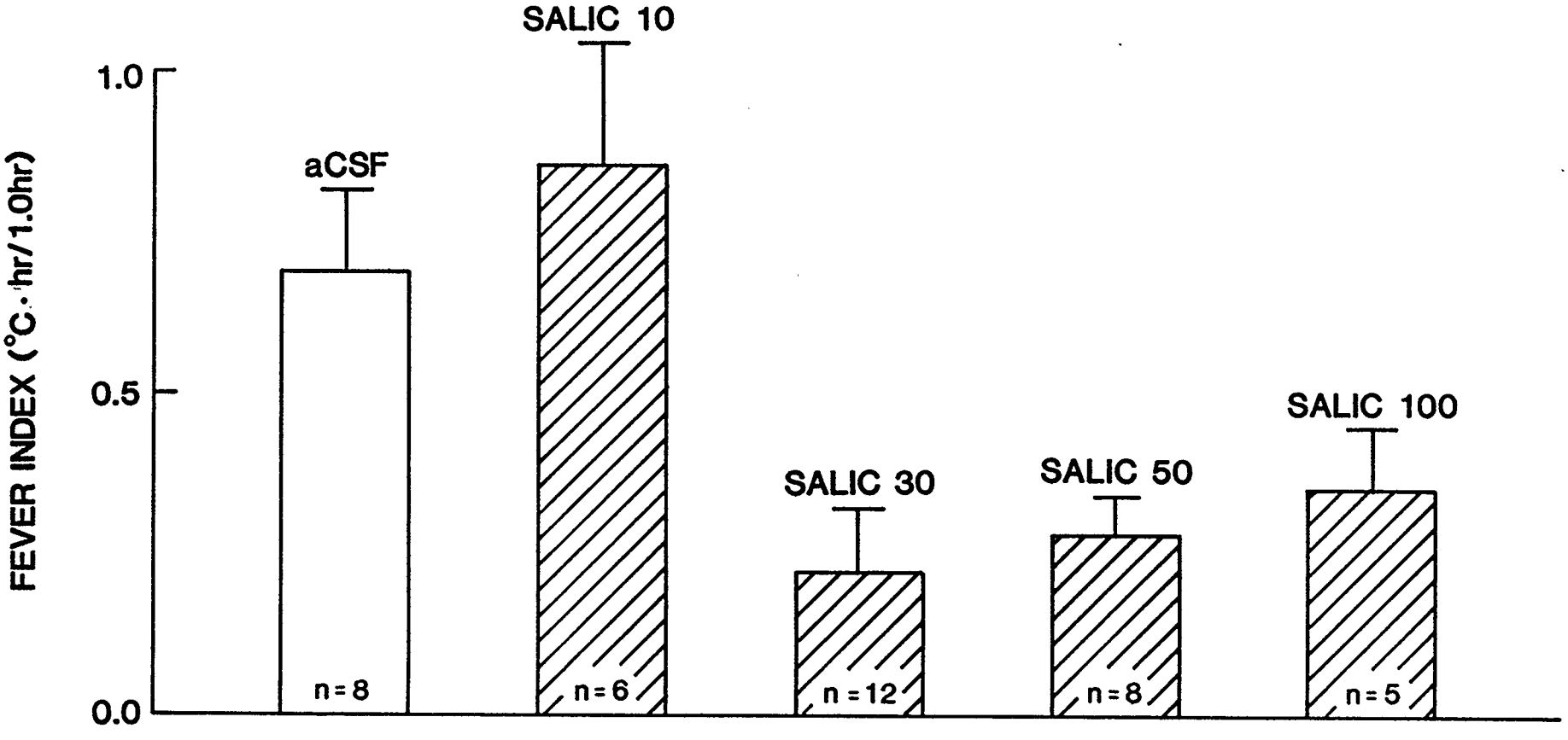

A
100.0 µg
B
50.0 µg
C
10.0 µg
2.0
1.0
0.0
-1.0
COLONIC TEMPERATURE - DEVIATIONS ROM BASELINE (°C)
-2
-1
0
1
2
HOURS

FIG. 11

The 1.0 h fever index (mean ± S.E.M.) following PGE_1 injection (icv) was not significantly different during infusion of 10.0 µg/µl sodium salicylate as compared to aCSF alone. The fever indices were significantly reduced during infusion of 50.0 µg/µl sodium salicylate ($p < 0.005$) and during 100.0 µg/µl sodium salicylate ($p < 0.05$) as compared to aCSF). In comparison to the index for 10.0 µg/µl sodium salicylate, the fever indices for 50.0 µg/µl and 100.0 µg/µl sodium salicylate were significantly reduced ($p < 0.001$). The fever index for 30.0 µg/µl sodium salicylate used in the previous experiment, included for reference, was significantly reduced when compared to either aCSF or the 10.0 µg/µl indices ($p < 0.001$). There were no significant differences between the fever indices for 30.0, 50.0 and 100.0 µg/µl doses of sodium salicylate.


SALIC 10
1.0
aCSF
FEVER INDEX (°C · hr/1.0hr)
0.5
SALIC 100
SALIC 30
SALIC 50
0.0
n = 8
n = 6
n = 12
n = 8
n = 5

A comparison of the results from the first experiments using 30.0 µg/µl sodium salicylate with the results from the present experiments using 50.0 µg/µl indicates that there is no significant difference between the two effective doses.

C. Discussion

The results suggest that the suppression of PGE-induced hyperthermia resulting from the infusion of sodium salicylate into the VSA may not depend on dosage. Alternatively, it could be that the dose-responsive nature of the effect exists within a very narrow range of concentrations. In the first case the results would suggest that the antipyretic effect of salicylate is an all or nothing effect. At some drug concentration lying between 10.0 and 30.0 µg/µl/hr, salicylate begins to have its effect within or near the VSA. In support of a dose-related effect within a narrow range is the observation that increased sensitivity of central nervous tissue usually requires a reduction in drug dosage for central versus peripheral administration (Cooper, 1965; Cooper et al., 1967; Stitt, 1986).

The three doses of sodium salicylate used in the present experiment represented what was considered to be an appropriate range of drug concentrations for the rat. Indeed while the inability and ability of the 10.0 µg dose and the 50.0 µg dose respectively, to block PGE-induced hyperthermia are straightforward, such is not the case with the 100.0 µg dose of salicylate. It is possible that within the rat brain the high dose of salicylate had a toxic effect. There are a number of reasons why this is possible.

1. The behavior of rats during the infusion of 100.0 µg/µl sodium salicylate was quite different from the behavior seen during infusion of the two lower doses. Typically, the rat would begin circling the cage as soon as the infusion was started. Whereas the rat was quite calm and unperturbed during aCSF, 10.0 µg and 50.0 µg infusions, it would try to escape the confines of the cage by jumping out or pulling itself over the edge during the higher dose. As well, the animal was more likely to be startled easily and respond to being startled with aggressive stances. Soon after termination of the infusion the rat would become calm once again.
2. The VSA seems particularly sensitive to pyretics and antipyretics as opposed to saline or other substances (Naylor et al., 1986a; Ruwe et al., 1986). For example, AVP push-pull perfused within the septal tissue is effectively antipyretic (Ruwe et al., 1985a), whereas a bolus injection of AVP into the VSA causes rats to display severe motor disturbances (Naylor et al., 1985b). A similar situation may exist in the case of low versus high doses of sodium salicylate. Whereas lower amounts of salicylate within the VSA were effectively antipyretic, large amounts of salicylate were somewhat disruptive.

For whatever reason, the mean temperature responses to the 100.0 µg dose of sodium salicylate were clearly not different from aCSF responses, indicating that the dose had a diminished antipyretic effect. It is known that high doses of salicylate can lead to paradoxical hyperthermia (Segar and Holliday, 1958; Flower et al., 1985). Paradoxical hyperthermia is thought to result because of the uncoupling of oxidative phosphorylation which, along with increasing oxygen uptake,

inhibits a number of ATP-dependent reactions (Packham, 1982; Flower et al., 1985). However, it should be noted that while salicylate-induced paradoxical hyperthermia does occur, the uncoupling of oxidative phosphorylation is difficult to induce in whole animals (Smith and Dawkins, 1971). Although paradoxical hyperthermia was not observed it is possible that the highest dose of salicylate (100.0 µg/µl) was eliciting other heat producing effects. The fever index for the 100.0 µg dose however, indicates that there was no difference between the 30.0, 50.0 and 100.0 µg doses. It would seem that while the 100.0 µg dose elicited responses which were quantitatively similar to the two other antipyretic doses (30.0 and 50.0 µg/µl), it still may be a dose which is too high for administration directly into the sensitive septal tissue.

Finally it has been suggested that drawing conclusions about mechanisms of action from dose-response data can be hazardous when the effect being studied is one that is distal to the stimulus or when the observed effect is a composite of several effects (Tallarida and Jacob, 1979). Infusion of sodium salicylate elicited an effect in core body temperature and it is difficult to estimate the number of events which occur between its effect in the septal region and the shift in temperature set-point within the posterior hypothalamus. What the present experiments do indicate is that there is a dose-related effect of salicylate within the VSA, in that one dose was ineffective while another dose was effective in bringing about antipyresis. Thus there is a narrow range of salicylate doses that, when infused within the neural tissue of the VSA, exert an effect on PGE-induced hyperthermia which is

quite different from its well known effects resulting from peripheral administration.

V. EVIDENCE THAT SEPTAL ADMINISTRATION OF SODIUM SALICYLATE DOES NOT LOWER NORMAL BODY TEMPERATURE

A. Introduction

In order to postulate that salicylate was antipyretic via an action on thermosensitive areas of the brain, an effect on thermoeffector pathways of the body would have to be overruled. For instance, one of the major arguments against a central antipyretic action on set-point originated from the observation that salicylates can be hypothermic in the nonfebrile rat.

In 1972 Satinoff reported that intraperitoneal salicylate injections reduced normal body temperatures of rats in a dose-dependent manner. Exposing female rats to a 5°C environment for one hour following an ip injection of sodium salicylate (60.0 - 300.0 mg/kg) resulted in significant drops in body temperature (up to 5.5°C) as compared to saline injected animals (Satinoff, 1972). The higher doses of salicylate (180.0 - 300.0 mg/kg) also lowered significantly the body temperatures of rats at room temperature. Francesconi and Mager (1975) achieved the same results as Satinoff. In addition they observed that salicylate did not effect oxygen consumption and did induce hyperventilation although that could not explain fully the changes in rectal temperature. Polk and Lipton (1975) showed that ip injection of salicylate (60.0 - 300.0 mg/kg) caused dose-dependent increases in the amount of time rats spent escaping heat. Hypothermia also has been noted when paracetamol or indomethacin are administered iv or ip in the rat (Feldberg and Saxena, 1974).

The hypotheses put forth to explain the hypothermic effect of i.p. salicylate in the rat have been varied. Satinoff postulated that prostaglandins might be released during cold stress as a way of maintaining normal body temperature and the blockade of prostaglandin synthesis by salicylates would therefore lead to hypothermia. However, since 1972 several experiments have shown that PGE levels of afebrile animals are low or undetectable (Coceani et al., 1983; Bernheim et al., 1980). Furthermore it has been shown that PGE levels in CSF do not change during cold exposure (Bernheim et al., 1980). Alternatively, Francesconi and Magner (1975) concluded that salicylate was hypothermic because of an increase in heat loss to the cold environment. This would be possible if salicylate enhances heat loss mechanisms such as vasodilation, hyperventilation or sweating.

In contrast are the majority of publications which have reported no effect of aspirin-like drugs on normal body temperature. Antipyretics administered orally, iv, ip, icv, or iontophoretically (in humans, monkeys, cats, rabbits, and rats) have been without effect on normal body temperatures. The common factor in reports of salicylate-induced hypothermia in the rat has been the peripheral administration of the antipyretic drug. The effects of centrally applied salicylates on rectal temperature of rats is an area which is in need of further investigation.

Throughout this research project sodium salicylate has been used extensively in the rat and so the question of possible hypothermia underlying the observed antipyresis was addressed. Specifically, the

present experiments were designed to observe the effect of septally administered sodium salicylate on normal body temperature of rats exposed to cold. If the sodium salicylate were interfering with heat production or enhancing heat loss then substantial decreases in body temperature would be expected.

B. Methods

To perform experiments in the cold alterations were made in the experimental procedure. The temperature of each rat was monitored continuously and recorded at 15 minute intervals beginning one hour before infusion and continuing for 90 minutes after the termination of the infusion. Rectal temperatures were measured during the following conditions of the experiment:

30 mins at ambient temperature of 21.0 ± 1.0°C

30 mins at ambient temperature of 10.0 ± 0.5°C

60 mins at ambient temperature of 10.0°C ± 0.5 - during Sodium Salicylate infusion

30 mins at ambient temperature of 10.0 ± 0.5°C

60 mins at ambient temperature of 21.0°C ± 1.0°C

These conditions were chosen as they are almost identical to those used by Satinoff (1972) except that under the present conditions the rats were exposed to cold for 1.0 hour longer than the rats in the former study. As well, the temperature of the cold room was 5.0 °C higher than that of Satinoff although this is the same temperature used in other cold exposure studies (Pittman et al., 1976). Data were analyzed using

the student's t-test for paired samples and results were considered significant at the $p < 0.05$ level.

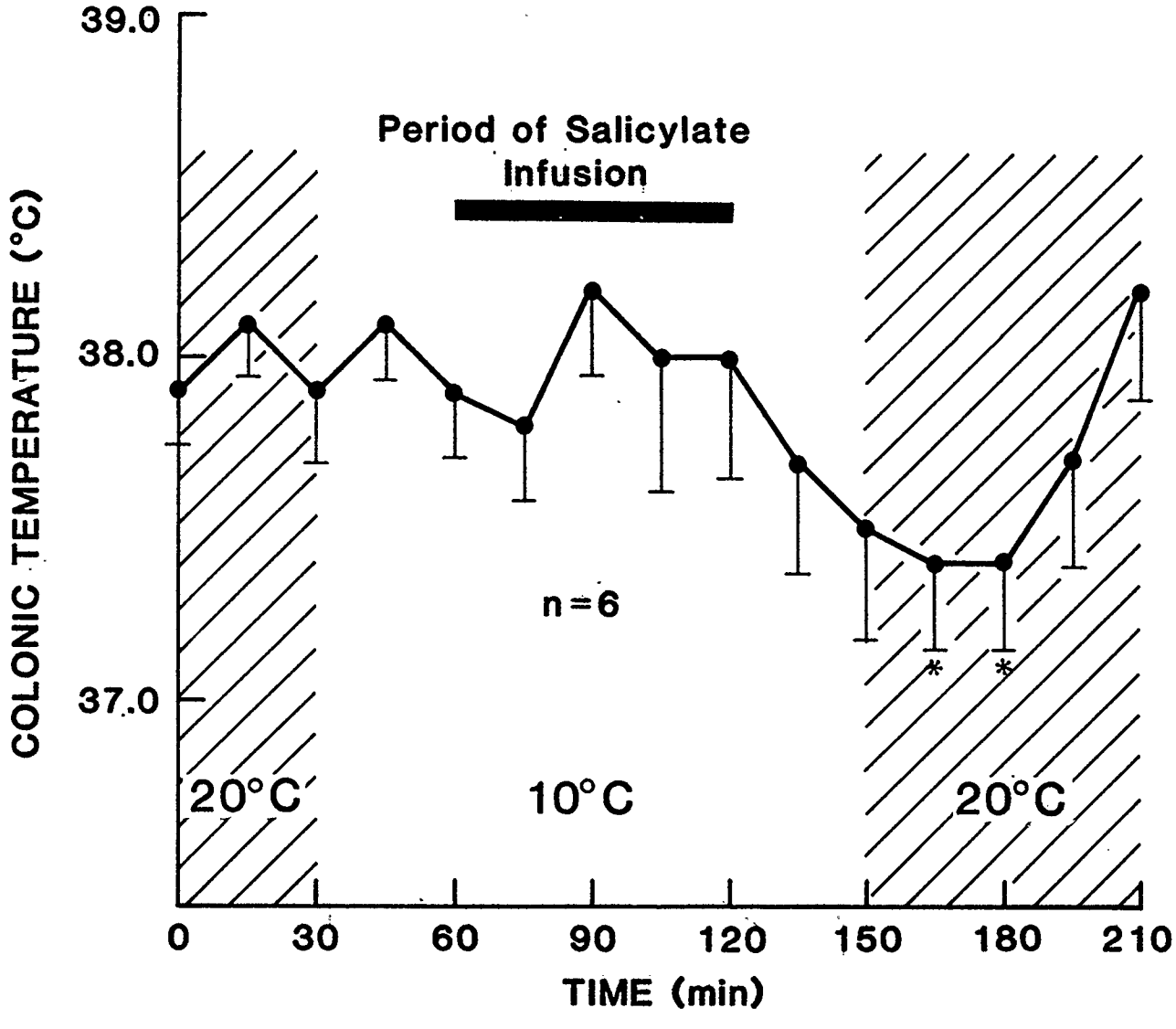
C. Results

Figure 12 illustrates the mean temperature responses of 6 animals to administration of sodium salicylate within the VSA during exposure to room and cold temperatures. Rectal temperatures did not decrease to any significant degree during the infusion of 50.0 µg/µl sodium salicylate (37.9 - 38.1 °C). Core temperatures were somewhat lowered 30 minutes after the termination of infusion (37.5 - 37.7 °C). When the rats were returned to room temperature the mean core temperature (37.4 °C) was significantly lower ($p < 0.05$) then starting or infusion core temperatures for approximately 30 minutes. Thereafter temperatures returned to normal (37.7 - 38.2 °C).

Thus, core temperatures at 45-60 minutes following the end of the infusion period were significantly lower than initial temperatures, infusion temperatures and temperatures shortly after infusion. The two low readings were 0.8 °C below the highest baseline temperature measured. The animals were not febrile, were seemingly healthy and regulating temperature normally.

FIG. 12

Mean temperature responses (± S.E.M.) in 6 animals. During a 1.0 h infusion of sodium salicylate (50.0 µg/µl) core body temperature was not significantly reduced from baseline temperatures. At 165 and 180 minutes, when the rats had been returned to room temperature, core temperature was significantly different from baseline and infusion temperatures ($p < 0.05$).


39.0
38.0
37.0
COLONIC TEMPERATURE (°C)
Period of Salicylate Infusion
n=6
*
*
20°C
10°C
20°C
0
30
60
90
120
150
180
210
TIME (min)

D. Discussion

The hypothermic responses reported by Satinoff (1972) after intraperitoneal injection of sodium salicylate were marked in that, core temperatures began falling almost immediately and continued to fall dramatically, reaching the lowest temperatures (-5.5 deviations from baseline temperature) 60 minutes after drug administration. Core temperatures reported in this experiment remained stable for the hour of infusion and only reached significantly lower levels (-0.48 to -0.5 deviations from baseline temperature) 45 after infusion ended. The mean deviation from baseline is much less marked than those reported after peripheral administration (Polk and Lipton, 1975; Satinoff, 1972). Furthermore, a 0.5 degree deviation in core temperature under these experimental conditions may not constitute hypothermia.

Based on previous research (Grundmann, 1969) it is known that iv doses of 300 mg/kg sodium salicylate in the afebrile rabbit resulted in an average of 1.7 - 3.3 mg/100 ml sodium salicylate appearing in cisternal CSF 90 minutes later. Hypothalamic levels of salicylate following administration of 300.0 mg/kg i.v. ranged from 1.22 - 1.92 mg/100 gm of tissue (Grundmann, 1969). These values correspond to nanogram per microlitre quantities of salicylate within brain tissue. The dose in this present work was 50.0 µg/µl which is a considerably higher dose of salicylate. It is interesting therefore that direct application of sodium salicylate to brain tissue in doses an order of magnitude greater than those doses resulting from peripheral administration would not produce hypothermia. Nevertheless, when using sodium salicylate appropriate control experiments should be done since

peripherally administered antipyretics in the rat, and in some cases the rabbit (Murakami and Sakata, 1975) and monkey (Chai and Lin, 1975) have disrupted, under certain conditions, the thermoregulatory abilities of these animals.

The purpose of the present experiments was to determine if sodium salicylate infusions within the VSA of the rat were causing significant or substantial decreases in core temperature which could explain the observation that septally administered sodium salicylate blocks exogenously induced PGE hyperthermia. If salicylate were causing hypothermia during the same time frame that it was antipyretic against icv PGE then the antipyretic results stated earlier in this thesis would have to be attributed to a disturbance of thermoeffector mechanisms or pathways. The lack of a hypothermic effect during infusion supports the theory that sodium salicylate is an antipyretic agent acting on central neuronal processes and not a hypothermic agent perturbing effector mechanisms.

Finally, the stability of the afebrile temperatures during sodium salicylate infusion seems to indicate a lack of toxicity to the brain tissue at the 50 µg/µl dose. Lower doses would presumably have no effect on afebrile temperatures as well. The effect of higher doses is uncertain at this time.

VI. SALICYLATE INFUSION WITHIN THE VENTRAL SEPTAL AREA REDUCES EFFECTS OF VASOPRESSIN ANTISERUM AND VASOPRESSIN ANTAGONIST

A. Introduction

The ability of sodium salicylate to interfere with the development of exogenously induced PGE hyperthermia was postulated to occur because of an enhancement of vasopressin release or receptor activity. The ventral septal area fulfills the requirements as the site of an endogenous antipyretic system of the brain (Veale et al., 1984; Kasting et al., 1982b; Kasting, 1980). It is known that vasopressin antiserum within the VSA enhances the febrile response to pyrogen and it is presumed to do so by antagonizing endogenous AVP action (Kasting, 1980; Malkinson et al., 1986). If salicylate enhances and AVP antiserum reduces the antipyretic effects of AVP within the septum then the infusion of both substances simultaneously within the VSA should negate the action of either substance. If salicylate is not enhancing the AVP antipyretic system then infusion of both salicylate and AVP antiserum should result in the enhanced pyrogen effect seen with antiserum alone.

Similarly, it is known that the V1 AVP antagonist, can block central vasopressin effects (Albers et al., 1986; Naylor et al., 1986d). Furthermore, within the septal area, this antagonist enhances the effects of a pyrogen challenge (Naylor et al., 1986b). Therefore, following the same argument as above, if sodium salicylate were to be enhancing the actions of endogenous AVP then sodium salicylate and AVP antagonist infused together into the VSA might be expected to result in a normal PGE hyperthermia. If there is no interaction between

salicylate and endogenous AVP then the effect of infusing both salicylate and antagonist should be the same as antagonist alone, that is, an enhanced fever.

B. Results

i. Sodium Salicylate and AVP Antiserum

During the infusion of 50.0 µg/µl sodium salicylate within the VSA, PGE_1-induced hyperthermia was suppressed to a significant degree ($p < 0.005$). Figure 13 indicates that rats receiving aCSF within the VSA responded to PGE_1 (icv) with a mean temperature increase of 0.85 ± 0.16°C at 30 minutes after PGE injection. The response to PGE was blocked by the infusion of sodium salicylate (0.10 ± 0.20°C at 30 minutes).

The infusion of AVP antiserum (1:10 dilution) resulted in a significantly enhanced PGE_1 hyperthermia compared to control infusions ($p < 0.005$ at 30 minutes). Figure 14 illustrates this enhanced response to PGE_1 as 1.34 ± 0.20°C after 30 minutes which is much higher then temperature responses during control infusions of 0.85 ± 0.16°C after 30 minutes.

FIG. 13

Mean temperature responses (±S.E.M.) in 6 animals. A 2.0 h infusion of aCSF alone did not significantly effect the PGE_1-induced hyperthermia (0.85 ± 0.16°C). However, the infusion of 50.0 µg/µl sodium salicylate into the VSA significantly suppressed (0.10 ± 0.20°C) the hyperthermic response to icv PGE_1 (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$; **** $p < 0.001$).

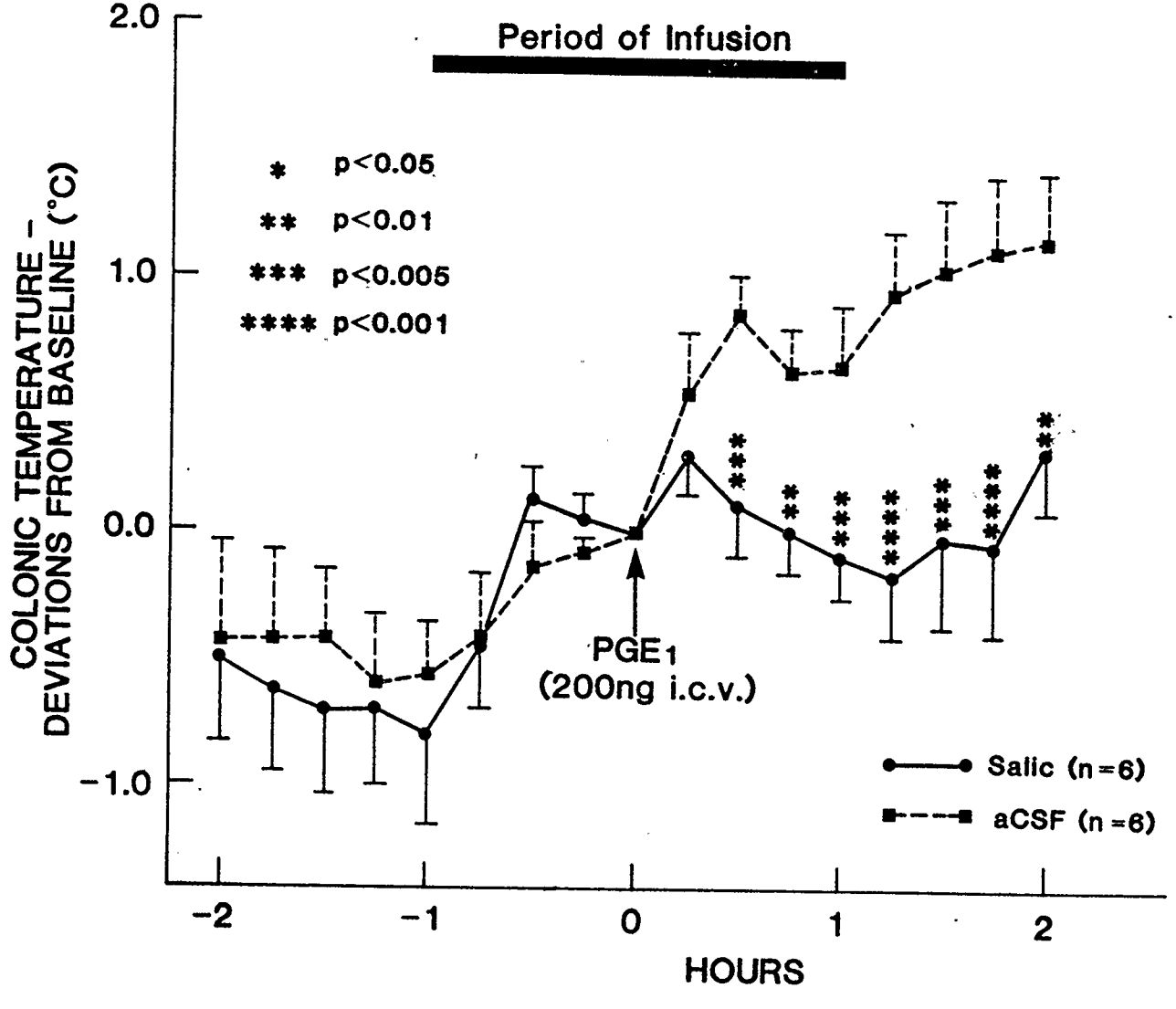
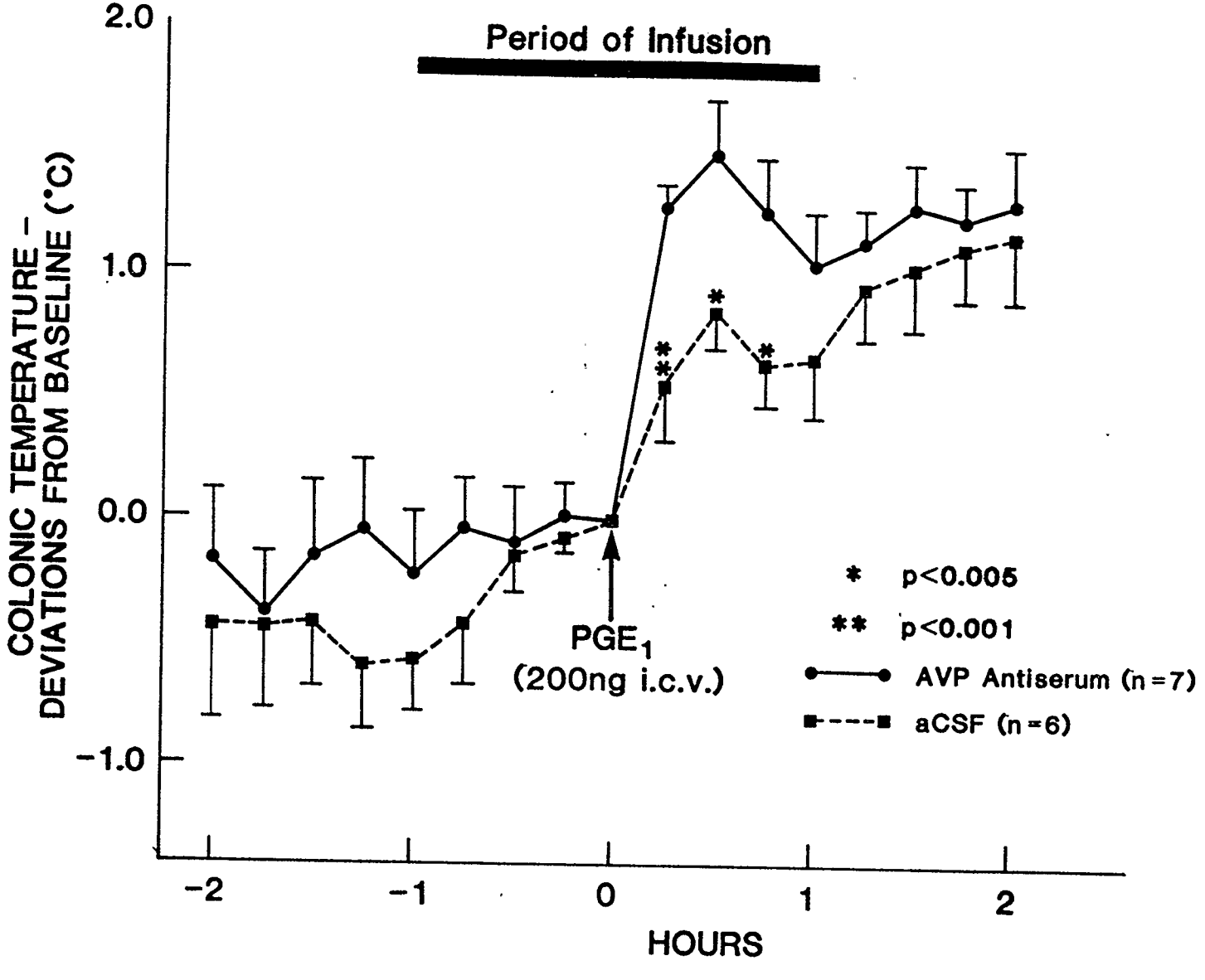

Period of Infusion
* p<0.05
** p<0.01
*** p<0.005
**** p<0.001
COLONIC TEMPERATURE - DEVIATIONS FROM BASELINE (°C)
2.0
1.0
0.0
-1.0
PGE1 (200ng i.c.v.)
Salic (n=6)
aCSF (n=6)
-2
-1
0
1
2
HOURS

FIG. 14

Mean temperature responses (± S.E.M.) in 7 animals. Compared to aCSF infusions within the VSA (0.85 ± 0.16°C), the 2.0h infusion of AVP antiserum significantly enhanced (1.34 ± 0.20°C) the PGE_1-induced hyperthermia (* $p < 0.005$; ** $p < 0.001$).


Period of Infusion
COLONIC TEMPERATURE - DEVIATIONS FROM BASELINE (°C)
2.0
1.0
0.0
-1.0
-2
-1
0
1
2
HOURS
PGE1 (200ng i.c.v.)
* p<0.005
** p<0.001
AVP Antiserum (n=7)
aCSF (n=6)

Observations made during the septal infusion of both sodium salicylate and AVP antiserum together indicate a PGE_1 hyperthermia similar to control levels. Figure 15 illustrates the mean temperature responses at 30 minutes after PGE_1 during the infusion of both drugs together (0.95 ± 0.25°C) or during the infusion of aCSF solution (0.85 ± 0.16°C). There were no significant differences between these two responses.

The fever index, measuring quantitatively the fever response for one hour following PGE_1 (icv), for each group is shown in Figure 16. It can be seen from this figure that the areas under the fever curve as compared to control infusions are: significantly less for sodium salicylate; significantly greater for AVP antiserum; and not significantly changed for both salicylate and antiserum together. The inset diagram indicates the area of tissue where bilateral infusions occurred which corresponds anatomically to the VSA.

FIG. 15

Mean temperature responses (± S.E.M.) in 6 animals. The response to PGE_1 (icv) during the 2.0 h infusion of both sodium salicylate (50.0 µg/µl) and AVP antiserum (1:10 dilution) together into the VSA did not differ significantly(0.95 ± 0.25°C) from the hyperthermic response during aCSF infusion (0.85 ± 0.16°C).

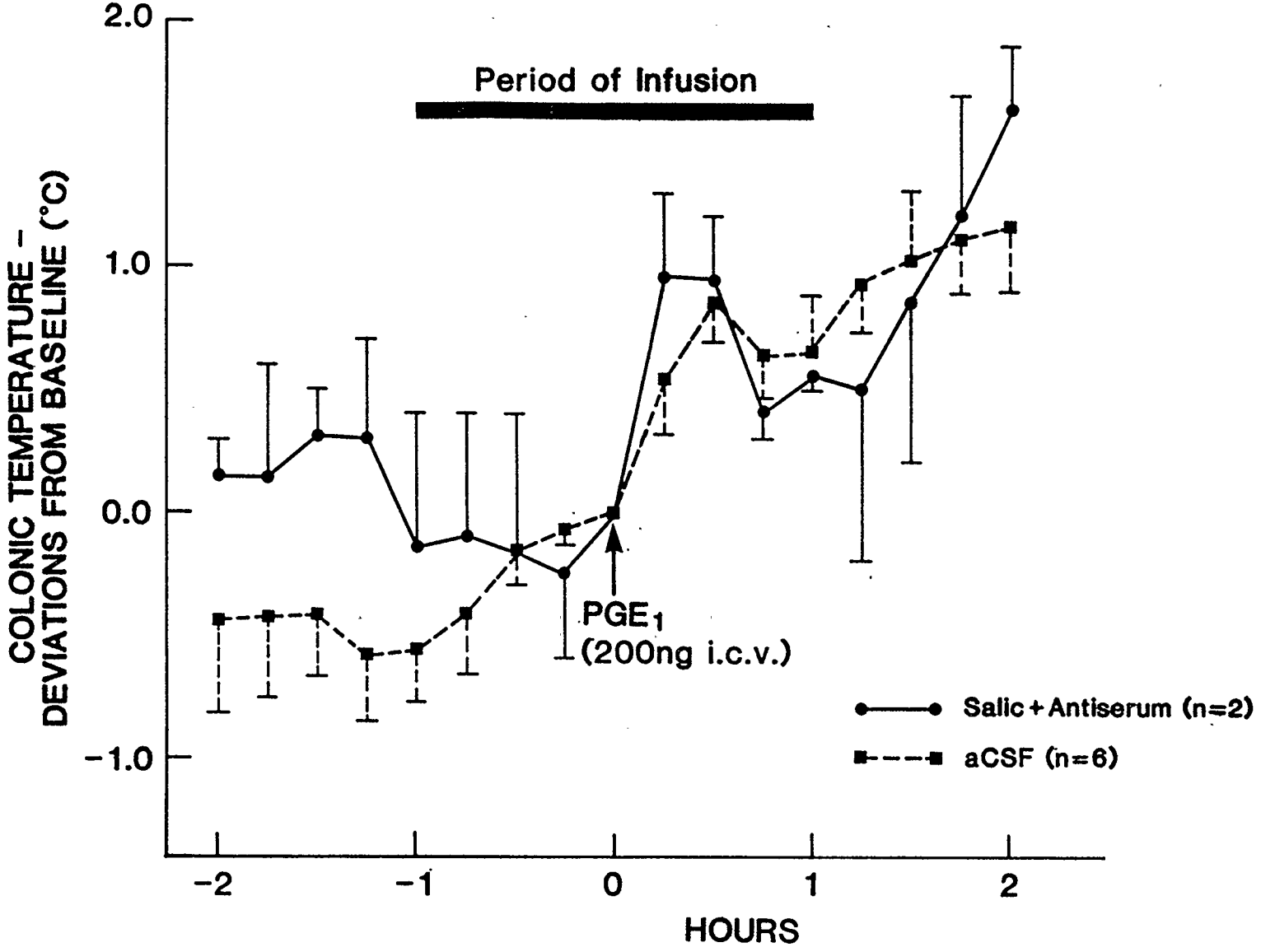
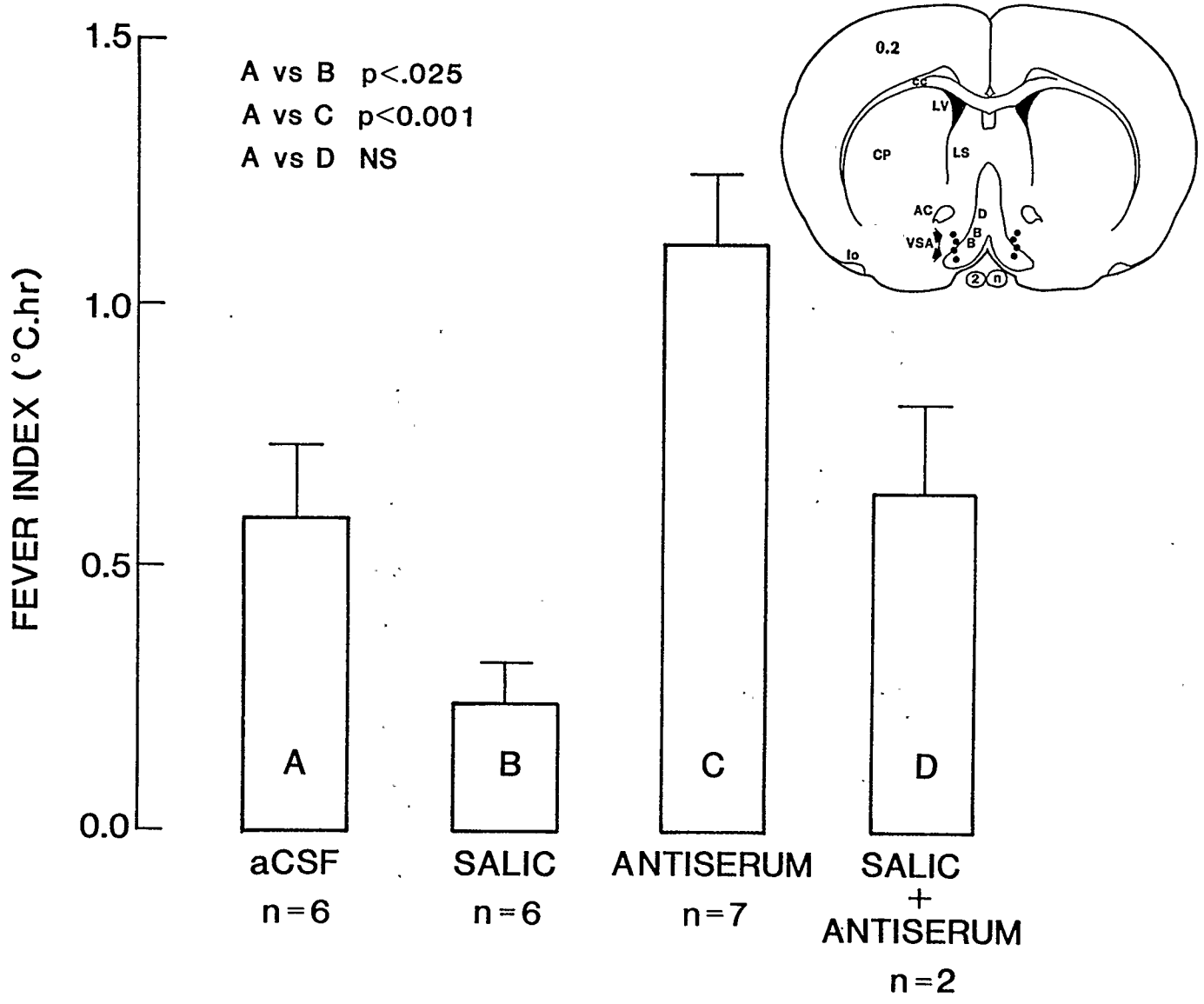

Period of Infusion
COLONIC TEMPERATURE - DEVIATIONS FROM BASELINE (°C)
2.0
1.0
0.0
-1.0
PGE1 (200ng i.c.v.)
Salic + Antiserum (n=2)
aCSF (n=6)
-2
-1
0
1
2
HOURS

FIG. 16

The 1.0h fever indices (mean ± S.E.M.) following PGE_1 injection (icv), as compared to aCSF, where significantly less for sodium salicylate ($p < 0.025$); significantly greater for AVP antiserum ($p < 0.001$); and not significantly different for both salicylate and antiserum together. (aCSF = 0.59 ± 0.14°C.h; sodium salicylate = 0.24 ± 0.08°C.h; AVP antiserum = 1.12 ± 0.13°C.h; both salicylate and antiserum = 0.64 ± 0.17°C.h). The inset is a schematic representation of a coronal section of the rat brain at 0.2 mm anterior to the Bregma. The filled circles indicate where bilateral infusions occurred which corresponds to the ventral septal area (VSA). Abbreviations are as in Fig. 6.


1.5
1.0
0.5
0.0
FEVER INDEX (°C.hr)
A vs B p<.025
A vs C p<0.001
A vs D NS
0.2
CC
LV
CP
LS
AC
D
VSA
B
B
Io
2
n
A
B
C
D
aCSF
n=6
SALIC
n=6
ANTISERUM
n=7
SALIC
+
ANTISERUM
n=2

ii. Sodium Salicylate and AVP Antagonist

The mean temperatures of 6 animals undergoing infusion of AVP antagonist [d$(CH_2)_5$ Try(Me)AVP] at two different doses (50.0 and 100.0 pM) in combination with sodium salicylate (50.0 µg/µl) indicated that the effect of AVP within the VSA was affected. The mean temperature responses (± S.E.M.) for doses of antagonist plus salicylate are shown in Figure 17 and are significantly different from the control infusions. Infusions of high doses of AVP antagonist and salicylate together resulted in rectal temperatures deviating from baseline by 2.18 ± 0.27°C at 30 minutes. This value was significantly greater ($p < 0.001$) than the control value (0.85 ± 0.16°C). Low doses of antagonist in combination with sodium salicylate resulted in temperatures deviating 1.15 ± 0.15°C from baseline in response to PGE which was not significantly different from the control response. Furthermore, there was a significant difference between the two doses of antagonist infused with sodium salicylate ($p < 0.01$).

The fever indicies for the antagonist treatments are illustrated in Figure 18. It can be seen that the areas under the fever curve as compared to control values are: significantly less for sodium salicylate; significantly greater for high doses of antagonist with salicylate; and not significantly different for the low dose of antagonist and salicylate together. A comparison of all fever indicies in this experimental series is provided in Figure 19.

FIG. 17

Mean temperature responses (± S.E.M.) in 6 animals. A 2.0 h infusion of both sodium salicylate (50.0 µg/µl) and AVP (V1) receptor antagonist (50.0 or 100 pM) together within the VSA resulted in an enhanced PGE_1-induced hyperthermia (1.83 ± 0.28°C) as compared to aCSF (0.85 ± 0.16) infusions (** $p < 0.01$; *** $p < 0.001$).

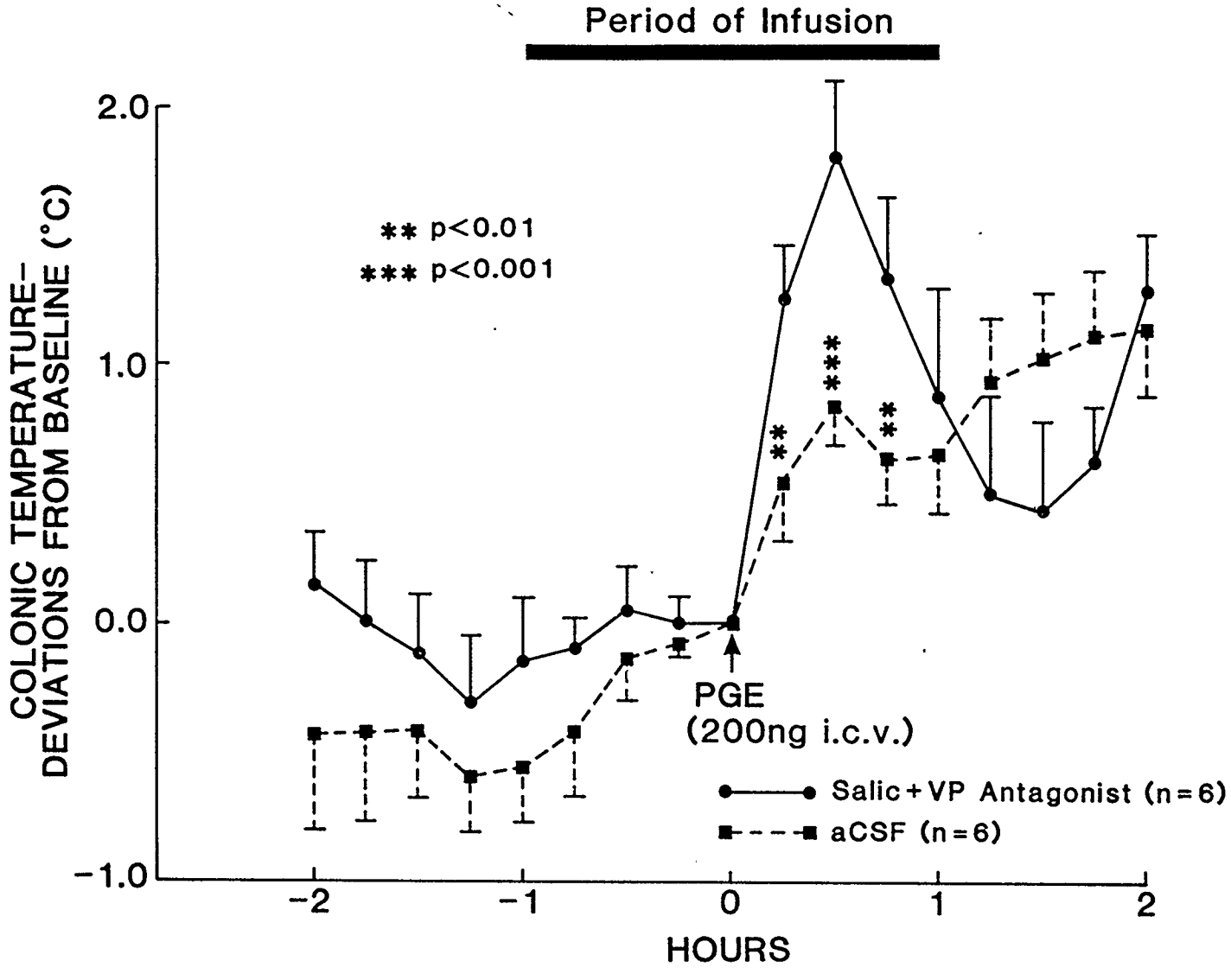
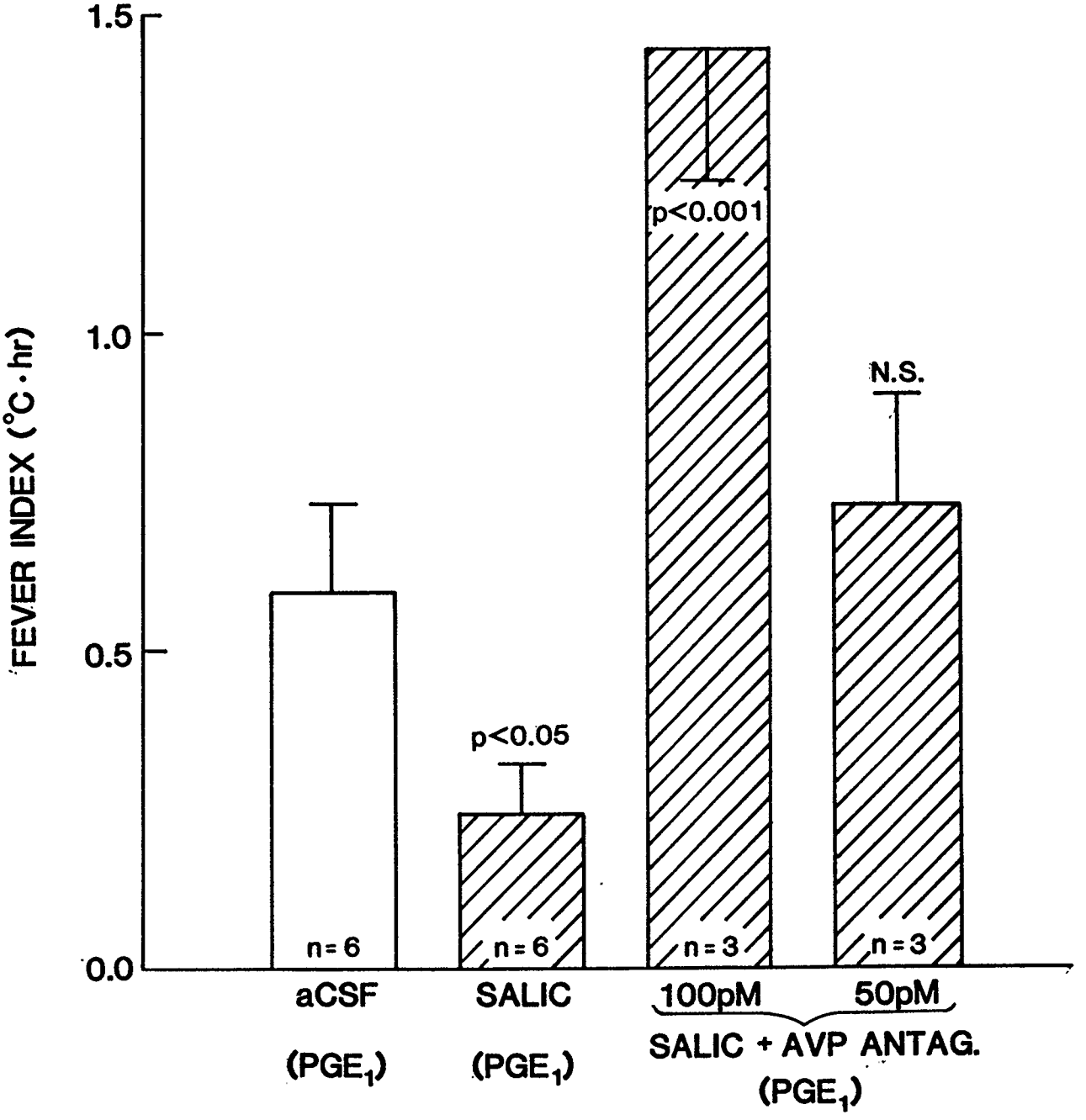

Period of Infusion
** p<0.01
*** p<0.001
COLONIC TEMPERATURE-
DEVIATIONS FROM BASELINE (°C)
2.0
1.0
0.0
-1.0
-2
-1
0
1
2
HOURS
PGE
(200ng i.c.v.)
Salic + VP Antagonist (n=6)
aCSF (n=6)

FIG. 18

The 1.0 h fever indices (mean ± S.E.M.) following PGE_1 injection of 18 animals. The fever index was significantly reduced ($p < 0.05$) during infusion of 50.0 µg/µl sodium salicylate (0.24 ± 0.08°C.h) as compared to infusion of aCSF alone (0.59 ± 0.14°C.h). The infusion of high doses of AVP (V1) receptor antagonist and salicylate resulted in significantly greater ($p < 0.001$) areas under the fever curve (1.45 ± 0.21°C.h) while the fever index for low doses of the antagonist and salicylate (0.73 ± 0.17°C.h) did not differ from that of aCSF. The index for both doses of antagonist together with salicylate (1.21 ± 0.21°C.h), not shown, was significantly greater than that of aCSF ($p < 0.005$).


1.5
1.0
0.5
0.0
FEVER INDEX (°C · hr)
p<0.001
N.S.
p<0.05
n=6
n=6
n=3
n=3
aCSF
(PGE1)
SALIC
(PGE1)
100pM
50pM
SALIC + AVP ANTAG.
(PGE1)

Histological examination of all the rat brains used for the above experiments confirmed that infusions were occurring at the level of the ventral septal area.

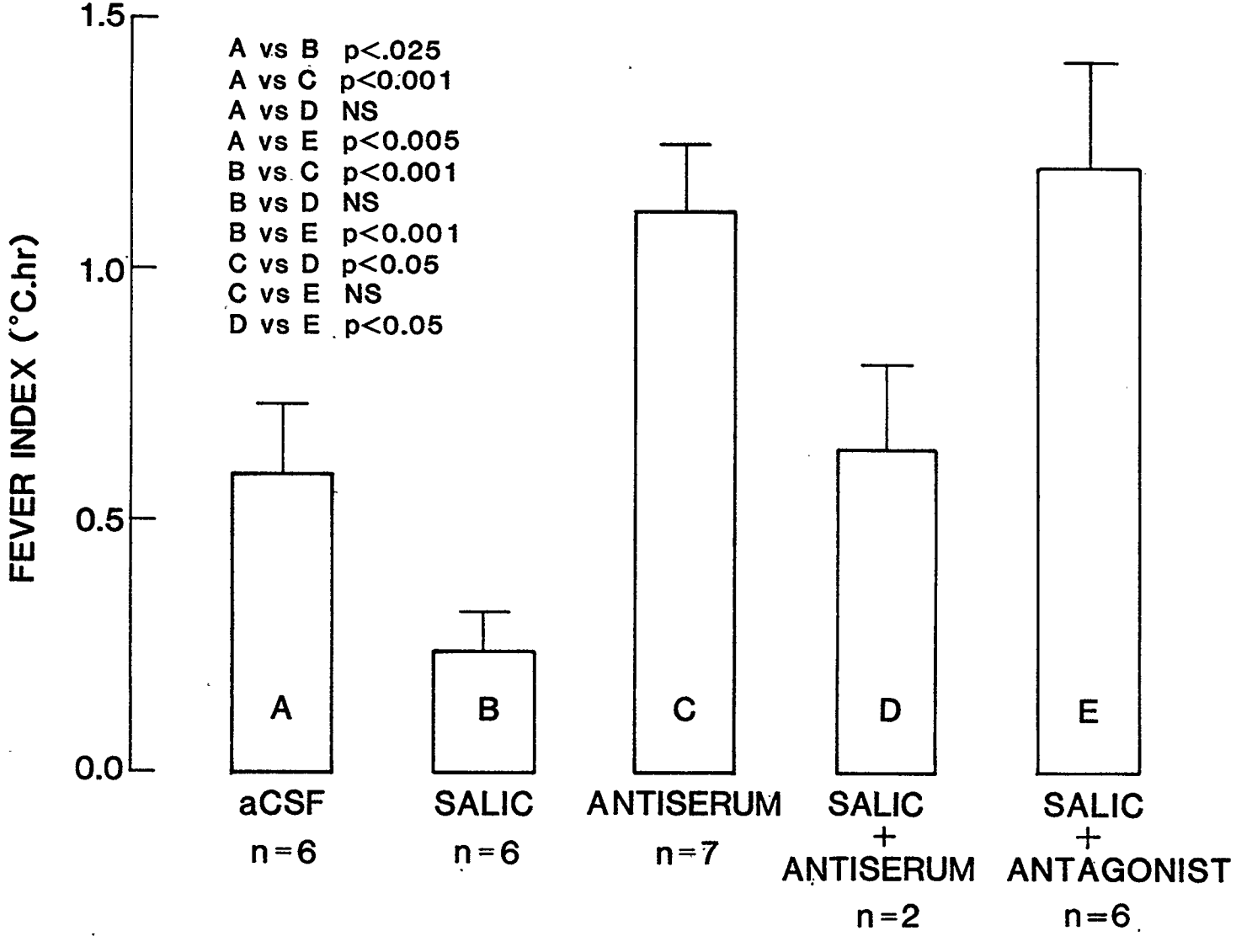
C. Discussion

The results of these experiments support the idea that exogenously administered sodium salicylate may be enhancing the antipyretic effects of endogenous AVP within the ventral septal area of the rat brain. The evidence can be summarized in the following manner:

1. Bilateral infusions of sodium salicylate (50.0 µg/µl) within the VSA significantly attenuated exogenously-induced PGE_1 hyperthermia.
2. Bilateral infusions of a specific AVP antiserum (1:10 dilution) within the same area significantly enhanced PGE_1 hyperthermia, supporting the idea that AVP is involved during fever and that AVP is adversely affected by the AVP antiserum.
3. Bilateral infusions of both sodium salicylate and AVP antiserum together reduce PGE_1-induced hyperthermia toward control levels.
4. Bilateral infusions of both sodium salicylate and AVP antagonist in low doses also reduce PGE_1 hyperthermia to control values.
5. Bilateral infusions of sodium salicylate and AVP antagonist in high doses result in significantly enhanced PGE_1 hyperthermia, suggesting that the high dose of antagonist was able to prevent any effect AVP might have had.

FIG. 19

Cumulative illustration of fever indices (mean ± S.E.M.) for all experimental groups in this section. Significant differences are indicated in the upper left corner. Types of treatment and number of animals treated are indicated on the abscissa.


FEVER INDEX (°C.hr)
1.5
1.0
0.5
0.0
A vs B p<.025
A vs C p<0.001
A vs D NS
A vs E p<0.005
B vs C p<0.001
B vs D NS
B vs E p<0.001
C vs D p<0.05
C vs E NS
D vs E p<0.05
A
B
C
D
E
aCSF
n=6
SALIC
n=6
ANTISERUM
n=7
SALIC
+
ANTISERUM
n=2
SALIC
+
ANTAGONIST
n=6

The fact that infusion of sodium salicylate alone can reduce hyperthermic responses to intraventricular PGE significantly but cannot do so in the presence of AVP antagonists, suggests that salicylate is interacting with AVP. Presumably if salicylate infusions did not involve endogenous AVP then AVP antiserum, which greatly enhanced PGE hyperthermia when infused alone, would not have any lesser effect when infused with sodium salicylate. The same argument would hold for the AVP (V1) receptor antagonist [$d(CH_2)_5$ Try(Me)AVP]. As reported, however, PGE-induced hyperthermia was brought to control levels by the infusion of sodium salicylate and AVP antiserum. PGE-induced hyperthermia was somewhat enhanced during the infusion of salicylate and the receptor antagonist. This can be explained in the following manner. If AVP receptor antagonists fully occupy the AVP receptor sites, then no extra release of AVP would cause antipyresis. However, if AVP is conjugated to its antibody in an amount which normally is released during fever, additional AVP released could act on empty receptors to cause some antipyresis. Regardless of the difference in effectiveness, the fever-enhancing effects of the AVP antiserum and antagonist were changed by sodium salicylate presence in the VSA.

Just recently, it has been shown that the AVP antagonist effectively blocks central AVP effects when administered within the brain. Naylor et al. (1986d) have shown that the antagonist can block AVP-induced hyperthermia in the AHPOA of the rat. In another set of experiments it was shown that the AVP antagonist used in this study greatly enhanced fevers due to IL1 when it was injected within the VSA

(Naylor et al., 1986b). Others have shown that within the medial preoptic-anterior hypothalamic area of the hamster this AVP antagonist can significantly inhibit AVP-induced flank marking (Albers et al., 1986). Thus it seems that central AVP actions can be upset by [$d(CH_2)_5$ Try(Me)AVP] administration. In these experiments [$d(CH_2)_5$ Try(Me)AVP] was able to undermine the ability of sodium salicylate to block PGE_1-induced hyperthermia.

While the present work does not prove that salicylate enhances AVP action, it is consistent with such a notion. It is especially attractive in light of the fact that the ventral septal area is rich in AVP-containing structures and has been shown to be intimately involved in endogenous AVP antipyresis. It is unclear at this point what the nature of the salicylate enhancement of endogenous AVP might be. A number of possibilities will be considered in the general discussion to follow.

VII. GENERAL DISCUSSION AND CONCLUSIONS

The purpose of the present research was to determine the effect of a classical antipyretic within the ventral septal area of the rat brain on the development of exogenously-induced hyperthermia. Sodium salicylate was infused slowly within the VSA before and after a PGE_1 challenge (icv). Surprisingly, sodium salicylate attenuated the hyperthermia which normally follows PGE_1 administration. If sodium salicylate's sole mechanism of action is inhibition of PGE synthesis and release (Vane, 1971) then the observed antipyresis should not have occurred. In order to discern the mechanisms by which sodium salicylate had such an effect, the site specificity of the response was investigated. It was found that areas more dorsal and caudal to the VSA did not respond in a similar manner to sodium salicylate administration. The site specificity of the salicylate effect thus was verified and provided the impetus for further investigations.

The VSA is known to be intimately involved in endogenous antipyresis, with arginine vasopressin being the endogenous peptide antipyretic (Ruwe et al., 1985b; Ziesberger, 1985). Push-pull perfustes samples indicate that AVP levels in the septal area correlate negatively with fever (Cooper et al., 1979) such that the extent of the fever is controlled (Veale et al., 1984). This has been verified by experiments which have administered AVP antiserum or AVP receptor antagonist within the VSA before pyrogen administration. The resulting fevers are significantly higher when AVP action is blocked (Malkinson et al., 1986; Naylor et al., 1986b). Recent work in the laboratory of Veale and Cooper has indicated that ablation of the VSA with kainic acid resulted

in an exaggerated fever to PGE (personal communication). The physiological observations supporting a role for AVP antipyresis within the VSA are supported by immunohistochemical and electrophysiological observations. The presence of AVP-containing fibres and AVP binding sites within the VSA has been confirmed in numerous investigations (Merker et al., 1980; Buijs, 1978, 1983; Junig et al., 1985; Baskin et al., 1983). Electrical connectivity from thermoresponsive cells of the VSA to major AVP containing neurons such as the PVN, BST and SCN has recently been established (Disturnal et al., 1985; Disturnal et al., 1986). Exogenous application of AVP to the VSA results in the attenuation of PGE-induced fevers as well as pyrogen fevers (Ruwe et al., 1985a, 1986; Naylor et al., 1985a, 1986a). These observations taken together indicate that endogenous AVP contained within the VSA plays an important role in thermoregulatory events of fever.

Sodium salicylate administered peripherally has been ineffective against PGE-induced fevers. The effect of central nervous system administration of salicylate against PGE-induced hyperthermia had not been tested. Since the VSA has now been implicated in fever modulation it seemed possible that sodium salicylate could be interacting with endogenous AVP thus bringing about the attenuation of PGE_1 hyperthermia.

A. Salicylate Enhancement of AVP Action

The possibility of an interaction between salicylate and endogenous AVP was tested using vasopressin antiserum and a vasopressin receptor antagonist in combination with salicylate administration. The blockade

of PGE-induced fever by sodium salicylate within the VSA was eliminated in the presence of the AVP antiserum or the AVP receptor antagonist. Large amounts of these antagonizing agents masked the sodium salicylate antipyresis completely. In contrast, smaller doses of antiserum or antagonist allowed the antipyretic effects of salicylate to remain noticeable. That is, the combined effect of salicylate and either antagonizing agent (in low doses) was such that PGE hyperthermia occurred at control levels rather than being reduced (salicylate only) or enhanced (antiserum or antagonist only). This supports an interactive relation between salicylate and endogenous AVP in the rat brain which may be responsible for the reduction of the PGE_1-induced hyperthermia.

Enhancement of drug action occurs when the presence of a second drug (salicylate) acting with the first (AVP) causes an increase in the effect (PGE blockade) produced (Tallarida and Jacob, 1979). It is possible that sodium salicylate is activating or enhancing the actions of the endogenous AVP antipyretic system which is then responsible for the observed antipyresis. Perhaps then, the infusion of aCSF does not block PGE_1 because it does not enhance or potentiate the effects of endogenous AVP.

Sodium salicylate could be sensitizing or causing an up-regulation of the septal AVP receptors. This possibility is supported by the fact that aspirin-like drugs can anchor themselves to the proteins of membranes and thus change the membrane structure and consequently its biological properties (Gryglewski, 1974). Such a change in the membrane

and/or its receptors could be to the benefit of the AVP action or to the detriment of other substances which may also be present.

Electrophysiological reports have shown that sodium salicylate applied to AHPOA tissue slices can reverse the effects of EP on the firing characteristics of neurons (Nakashima et al., 1985; Hori et al., 1984). Even though salicylate application in the absence of EP does not significantly alter the firing rate of neurons, these authors have reported a tendency for salicylate to increase firing rate in many thermosensitive and thermally insensitive neuronal units. Thus the possibility exists that salicylate is stimulating the AVP-containing synapses of the VSA (Buijs and Swaab, 1979) to a slight degree and this might be sufficient to enhance AVP release.

B. Salicylate-Induced Changes in Ion Conductance

Alternatively, salicylate may be causing changes in various ionic conductances and therefore changing the responsiveness of VSA neurons. It is known that salicylate facilitates potassium exchange (at least between blood and CSF, Cameron, 1968) by poisoning active processes which restrain K^+ exchange. In molluscs, salicylate application (10 - 100 mM) caused an immediate, dose-dependent, and reversible hyperpolarization of the resting membrane potential of buccal ganglion cells by increasing K^+ permeability and decreasing Cl^- permeability (Barker and Levitan, 1971). The authors suggested that the negatively charged salicylate molecules absorbed to the neuronal membrane so that ion conductances were altered (Levitan and Barker, 1972). These effects of salicylate would result in a decrease in the output from and the

input to the affected neurons. Hyperpolarization of thermosensitive VSA cells by salicylate infusion would render them less sensitive to thermal inputs which would reduce amount of AVP which is being released within the VSA during fever. In light of the established involvement of AVP in fever this does not seem a likely mechanism of action. While it is clear that salicylate alters ionic mechanisms between blood and CSF and in molluscan neurons, conclusions about such alterations in mammalian cells in the VSA can not be made at this time.

C. Salicylate is Blocking Further PGE Release

One question which comes to mind is whether PGE_1 given icv induces further release of endogenous PGE_1 in the brain. In this case, the action of sodium salicylate within the VSA could still be explained as inhibition of PGE synthesis and release. However, there have been reports which cast doubt on the notion that PGE injection evokes PGE release. In fact, reports have indicated that PGE levels do not rise in response to various experimental manipulations (Coceani et al., 1983). For example, tissue damage caused by sampling of CSF of cats increased levels of thromboxane B_2 (TXB_2) but not levels of PGE_2 in perfusate samples. As well, basal levels of neither TXB_2 nor PGE_2 changed when CSF withdrawl rate was altered or when repeated samplings occurred in the same animal (Coceani et al., 1983). These observations support the notion that endogenous PGE release does not readily or necessarily occur during manipulations within the brain.

Furthermore, if salicylate were blocking endogenous PGE synthesis caused by PGE given icv, then the 10.0 µg dose of salicylate also should

have resulted in decreased PGE-induced hyperthermia. Such was not the case even though the 10.0 µg dose is higher than doses given into the AHPOA which have been antipyretic against intravenous pyrogen fever (Cranston and Rawlins, 1972).

Two recent papers have given evidence which is not consistent with the notion of PGE inducing further PGE release. Evidence from in vitro experiments suggests that PGE release from the hypothalamus requires Ca^{++} influx to nerve terminals (Ojeda and Negro-Vilar, 1984) and evidence from experiments on rabbit cervical ganglion cells indicates that exogenous PGE inhibits Ca^{++}-dependent potentials through a decrease in Ca^{++} conductance (Mo et al., 1985). Taken together these results suggest that exogenous PGE might decrease Ca^{++} conductance at cell membranes which in turn would decrease or inhibit endogenous PGE release. Thus a kind of negative feedback loop may exist within the brain which would prevent PGE from inducing further endogenous PGE release.

D. Summary

Experiments carried out previously have provided evidence that aspirin-like drugs are antipyretic through their ability to inhibit PGE synthesis and release. The work presented in this thesis provides new information in that an alternate mechanism of action for sodium salicylate within the ventral septal area of the rat brain could be in effect. The time course of the salicylate-induced reduction of PGE hyperthermia, that is, during the first half hour following PGE injection, suggests that an action other than inhibition of the

cyclooxygenase enzyme must be involved. The possibility that salicylate was disrupting thermoeffector mechanisms was investigated by exposing rats to cold during salicylate infusion within the VSA. The rats did not become hypothermic during the course of salicylate infusion which suggested that thermoeffector mechanisms were unaffected by salicylate (in the manner of a true antipyretic). In an effort to explain salicylate's blockade of PGE-induced hyperthermia it was postulated that endogenous AVP within the VSA was playing a vital role. The attenuation of the salicylate effect on PGE hyperthermia by simultaneous infusion of salicylate and one of two AVP antagonizing agents was taken as supporting evidence for the involvement of AVP in the salicylate blockade of exogenous PGE. The results reported herein have indicated that sodium salicylate can exert antipyretic actions within the CNS which are not related to its ability to inhibit the cyclooxygenase enzyme and thus PGE synthesis and release. The possibility that salicylate was interacting with endogenous AVP within the VSA was investigated and supportive evidence was obtained. Generally then, there are several approaches which now could be used to pursue the question of an interaction between exogenous sodium salicylate and endogenous AVP within the VSA.

The use of AVP receptor antagonists would help to clairfy whether an interaction between salicylate and AVP is occurring at the AVP receptor sites of the VSA. Electrophysiological recordings made from a septal tissue slice preparation would help to clarify any direct membrane effects that salicylate might be having. For instance, the effects of salicylate on firing rates of cells in the AHPOA slice have

been investigated. As well, neuronal properties of invertebrate ganglia have been monitored and changes due to salicylate application were reported. However, the characterization of neuronal cell responses and changes in cell membrane properties of cells within the mammalian VSA during antipyretic administration have not been investigated. If neurophysiological changes occur during salicylate administration, such changes may lead to ideas about the mechanism of action of salicylate within VSA. Release studies, investigating the possibility that salicylate is inducing AVP release, could provide another means of addressing the question more directly. One method which has proven useful is push-pull perfusion followed by an assay for AVP. Basal levels of AVP could be compared to AVP levels following salicylate administration. Release studies may provide a more direct indication of the effects of salicylate on VSA cells.

Other investigations could focus on the dose-related nature of the sodium salicylate blockade of PGE-induced hyperthermia. Doses lying between the ineffective 10.0 µg/µl dose and the effective 30.0 µg/µl dose of salicylate could be tested and a dose-response curve constructed. As well, antipyretics such as aspirin, indomethacin or acetaminophen should be tested since the lipid solubilities (and the antipyretic potency) varies markedly between them and there may be a difference in their effect on PGE given icv. Any differences, in turn, may give some indication as to the mechanism of action by which sodium salicylate suppressed PGE-induced hyperthermia.

In conclusion the results reported in this thesis underscore the large number of interactions that seem to manipulate the environment of the brain. The observation that a classical antipyretic exerts effects that previously were unknown reminds one of how little is know about the brain and its complex interactions with drugs.

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