THE EFFECT OF AUGMENTATION OR V_1 BLOCKADE OF PERIPHERALLY RELEASED AVP DURING BACTERIAL INFECTION

By

Jeanne Ann Korn

B.Sc., Northern Arizona University

A THESIS IN PARTIAL FULFILLMENT

OF THE REQUIREMENT FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Physiology)

We accept this thesis as conforming

to the required standard

THE FACULTY OF GRADUATE STUDIES

October 1989

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Physiology

The University of British Columbia
Vancouver, Canada

Date Oct 13, 1989

ABSTRACT

Research into the effects of fever has recently had a resurgence of interest with the finding that fever has a definite beneficial role in host defense during bacterial infection in lizards, frogs and fishes. Studies in mammals have not been so definitive. For example, prevention of fever by non-steroidal anti-inflammatory drugs has reduced survival in rabbits but artificially increasing the febrile response in rats has decreased survival. Few studies, however, have sought to determine the important contribution of other physiological variables to the febrile response, focusing rather on simply the rise in temperature.

Arginine Vasopressin (AVP) has been shown to be released both centrally and peripherally in high quantities during fever. Release of AVP into the ventral septal area (VSA) of the brain serves as an endogenous antipyretic preventing potentially harmful body temperature increases. The possible role peripherally released AVP plays during infection has not been clearly established, thus these studies were conducted to make a preliminary assessment of that role.

Previous experiments have demonstrated that peripherally released AVP may have important functions other than the maintenance of water balance that might contribute to host defense against infection. These include maintenance of cardiovascular function, stimulation of glycogenolysis and gluconeogenesis in the liver, stimulation of phagocytic activity in the reticulo-endothelial system and increases in corticosteroid concentration in the blood.

In the experiments reported here, augmentation of the normal AVP response during infection due to gram negative bacteria, altered the thermoregulatory response. There was a pronounced hypothermic phase occurring at 12 hours after

injection of 0.5 ml live E. coli bacteria. Additionally, fever at 24 hours was slightly diminished in the AVP infused rats. However, AVP augmentation did not significantly alter the survival of the rats as compared to saline controls.

Blockade of endogenously released AVP at the V_1 receptor prior to a single injection of live E. coli bacteria, resulted in an increased febrile response. The difference in body temperature from baseline 24 hours after injection was greater in the V_1 antagonist-infused rats. Recovery time was increased in the antagonist-infused rats indicating a possible role of AVP in the acute phase reaction to bacteria. Again, survival did not differ.

In the longer term infection model, which involved repeated injections of bacteria, V_1 receptor-antagonism did not significantly alter the course of infection as measured by body temperature. There were some differences between the two groups particularly in the first four hours, as the antagonist-infused rats had a pronounced hypothermia, again suggesting some involvement with the acute phase reaction.

The data from the long-term infection model suggest that other compensatory mechanisms are able to replace the contribution of AVP acting at the V_1 receptor or that endogenous AVP release is increased to such an extent as to replace the antagonist at the receptor site. A systematic series of experiments combining and eliminating other hormones released during sepsis would be required to determine the possible synergism or redundancy these hormones have during infection.

In summary, AVP clearly has some involvement in the cascade of events involved in the defensive reaction to infection, but AVP acting at the V_1 receptor does not play a crucial role in the outcome utilizing this infection model.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

It is a pleasure to be given the rare opportunity to acknowledge and thank the people who have inspired and helped me.

First I would like to thank Norman Kasting for giving me the opportunity, for taking me on as a graduate student sight unseen. Also for showing me the model of a true scientist, involved in it because of a fascination which can't be helped.

I would like to acknowledge the patient instruction and ability for scientific banter which is the gift of Marshall Wilkensen.

I would like to extend an enormous thanks to John and Joe and Mary and Zaira without whom not much would get done in the Physiology department.

I would like to thank Ray Pederson for pulling for me from the beginning all the way through.

Finally, I would like to acknowledge my parents, Bonnie and Jerry Korn, for being forever supportive of anything I've decided to do.

INTRODUCTION

A. Thermoregulation

In 1808 James Currie wrote that, "If a definition of life were required, it might be most clearly established on that capacity, by which the animal preserves its proper heat under the various degrees of temperature of the medium in which it lives." The fact that all life- sustaining, enzyme-catalyzed biochemical reactions occur within a narrow temperature range confirms his observation. If organisms were in no way capable of maintaining a fairly constant internal body temperature they would quickly become extinct. The ability of an organism to thermoregulate whether behaviorally, physiologically or both is phylogenetically conserved from plants to man (Kluger, 1979). This strongly suggests some adaptive function.

Thermoregulation of animals can be said to fit into two separate classifications: Poikilotherms display a relatively wide range of body temperature and generally use behavioral means to minimize this range. Homeotherms, on the other hand, have a narrow zone of thermoregulation, and achieve this through both physiological and behavioral means. They have the ability to activate either heat loss or heat gain effector mechanisms accordingly. Temperature regulation of this variety is advantageous because the animal can "afford" to exploit a broader range of environments. It is also very energetically expensive, and the cost increase is directly proportional to the degree of thermoregulatory capability, that is, mammals have the most metabolically expensive machinery for their sophisticated temperature regulation.

In order to regulate body temperature at any given range, heat gain (metabolic rate + heat gained from the environment) must equal heat loss. (Heat gain=

Heat loss). Heat exchange with the environment occurs in four ways: Conduction is the loss of heat from a hotter to a colder object with which it is in contact, and requires a thermal gradient. Convection is the loss of heat through moving air or water. Evaporation is based on the energy required to change a liquid into a gas (heat of vaporization). Radiation is heat given off from a radiating body to the environment. These mechanisms hold true for both poikilotherms and homeotherms, but can be regulated physiologically by homeotherms, thereby maintaining homeostasis despite fluctuating environmental conditions.

B. Fever

Fever has long been used as a diagnostic tool indicating inflammation and infection. Since the beginning of recorded history it has represented both a horror and fascination in the minds of men. Hippocrates' humoral theory of fever suggested that it was caused by an imbalance of one of the four "humors" in the body, specifically the over production of yellow bile. This theory dominated thought on fever through the 18th century. In the late 1800's, Claude Bernard advanced his theory of body temperature regulation, wherein heat gain is offset by heat loss via the autonomic nervous system. This fits into his covering theory of homeostasis: maintenance of the body by a predetermined "status quo". In 1888, William Welch wrote a classic treatise on fever utilizing his experiments in microbiology and pathology concluding that elevated body temperature might not be harmful per se and might be, in fact, beneficial to the host (Atkins & Bodel,1979).

Fever is defined as an elevation of body temperature above normal that is maintained and defended (Stitt,1979). Specifically, it is caused by the action of pyrogens on the hypothalamic thermoregulatory neurons which determine the "set-point" body

temperature. This temperature is maintained by the autonomic nervous system via heat-loss and heat-gain effector mechanisms.

The precise mechanism whereby this shift in hypothalamic set-point occurs is not clearly understood, however, there are some generally accepted theories regarding the pathway involved in the normal development of fever. Initially, an infectious agent or pyrogen enters the blood stream and stimulates macrophages and monocytes to release an endogenous pyrogen (EP).

This endogenous pyrogen has been looked at extensively and may consist of not one but several substances. The stimulated cell may release interleukin-1 (IL-1), tumor necrosis factor, or interferon- (INF-), as well as prostaglandins (PG's), and putative endogenous pyrogens continue to be discovered. All of these substances have been shown to cause fever when injected into the bloodstream.

After entering the bloodstream, they are thought to act on the reticulo-endothelial system and the brain to release molecules that mediate the message and effect the response to infection such as fever (Stitt,1985). With the possible exception of the PG's these substances are of high molecular weight and there is no evidence that they have the ability to cross the blood brain barrier to gain access to the preoptic anterior hypothalamus (POAH) that is the likely brain locus for thermoregulatory control, thus a secondary mediator would be required.

A possible location where these molecules could gain access to the brain is a circumventricular organ known as the organum vasculosum lamina terminalis (OVLT). The capillaries lining this area of the brain are fenestrated and may therefore provide access of the (EP), which in turn stimulates brain neurons to release a mediator that translates the message to the POAH neuropile.

The identity of the mediator remains an open question, however; the prostaglandins of the E series seem to be likely candidates. The definitive role of the PG's is controversial, but good evidence in favor of a role is provided by the fact that PG levels increase in the cerebrospinal fluid during fever and that known prostaglandin synthesis inhibitors reduce fever while leaving an afebrile body temperature intact (Vane, 1971, Feldberg & Gupta, 1973).

Although it is unknown whether fever is in itself essential to the host's survival during infection, it has been demonstrated to be important in reptiles (Kluger, 1975), amphibians (Kluger, 1977) and fish (Reynolds et al, 1978). In his classic study utilizing the lizard Dipsosaurus dorsalis, Kluger demonstrated that when injected with a bacterial pyrogen the lizards sought out a higher environmental temperature in order to develop a fever and that when prevented from doing this their survival was decreased (1975). As he eloquently put it:

> "If fever did not have an adaptive function then it would be unlikely that this energetically expensive phenomenon would have persisted for millions of years in at least six orders of mammals and vertebrate-classes from the bony fishes through the birds".

Studies in mammals include a study on rabbits by Kluger and Vaughn (1978) that demonstrated that fever height up to 2.25°C was correlated with an increase in survival but that fevers over 2.25°C were associated with a decrease in survival. Vaughn and coworkers (1979) later showed a decrease in survival rate of rabbits infused with the antipyretic drug sodium salicylate prior to injection of live bacteria. Banet (1979) demonstrated a similar phenomenon in rats. When the hypothalami were cooled the normal development of fever to bacterial infection could be enhanced. Abnormally high fevers were associated with a decrease in survival, verifying the work by Vaughn et al (1979).

The widespread clinical use of antipyretics on the other hand leads one to the assumption that reduction of fever is not harmful and questions the adaptive value of fever. However, one might challenge this as antipyretics are rarely used prior to the development of fever. It may be that the events associated with the initial temperature increase of the febrile process are critical. Banet (1981) suggests that the metabolic rate increase associated with the rising phase of fever may be a significant contributing factor, but that is by no means the only event occurring.

C. Metabolic Response to Infection

An invasion of an infectious agent into the host organism causes not only fever but a whole cascade of events involved in the defensive reaction. Some, such as those directly associated with the immune response; eg. leukocyte migration, mobilization of phagocytes, and production of antibodies are specific and their adaptive function is obvious. Others are more generalized, such as fever and hormonal changes associated with infections, and their function remains to be clearly defined. It is apparent, however, that the host defenses that come into play during illness are very much an integrated process and looking at a single aspect of it is like looking at a single organism in an intricate ecosystem.

The cardiovascular changes that occur during infection are numerous. One of the most obvious is peripheral vasoconstriction causing the characteristic pallor observed in illness. This serves to decrease heat loss to aid in establishing a fever. Other responses include redistribution of blood flow, which increases in hepatic, splanchnic and renal vascular beds (Bennett & Beeson, 1950). Mean arterial pressure is decreased in the acute phase reaction to infection (Bennett & Beeson, 1950). Heart-rate increases but is not directly correlated with increases in body temperature. (Cooper, 1971, Bennett & Beeson, 1950).

Metabolic changes include: a decrease in liver glycogen with an associated transitory increase in plasma glucose (Bennett & Beeson, 1950), an increase in glucose turnover, and increased gluconeogenesis (Hargrove,et al., 1988).

Hormonal changes in the plasma are extreme and only a brief summary will be stated here. There are well documented increases in circulating glucocorticosteroids, and catecholamines (Egdahll, 1959). Thyroid stimulating hormone (TSH) and growth hormone (GH) both increase in humans but decrease in the rat (Kasting & Martin, 1982). There is an increase in glucagon and insulin (Curnow et al, 1976). Renin-angiotensin is increased as is aldosterone (Bennett & Beeson, 1950). Oxytocin and vasopressin are both increased (Kasting, 1987).

D. Arginine Vasopressin (AVP)

Vasopressin is a nonapeptide synthesized principally in the paraventricular, supraoptic and suprachiasmatic nuclei of the hypothalamus. AVP then travels down long axons to be released into the peripheral circulation from the neural lobe of the pituitary and also into the median eminence of the portal circulation. As will be discussed later, however, AVP containing neurons with projections throughout the brain suggest that AVP may be acting as a neurotransmitter or neuromodulator. (Buijs et al, 1978)

Centrally-released AVP during febrile episodes has been proposed to be an endogenous antipyretic preventing potentially harmful fevers from developing. Evidence for this initially came from experiments on the development of fever in newborns. The fact that neonates have an inability to respond to pyrogens with the development of fever has been known for some time. Two species: guinea pigs and lambs, were chosen

for further investigation in this area because of their mature thermoregulatory systems at birth.

In 1974 Pittman and coworkers found that lambs did not respond with fever to injection of bacterial endotoxin at 4 hours or at 60 hours after birth, but if injected at 4 hours and then again at 60 hours they were able to develop fevers. This indicated a sensitization process might be involved perhaps through the immune system. It was also observed that the near or post-partum ewe was unable to develop fever in response to pyrogen (Kasting et al, 1978).

The question was raised as to whether the inability of newborns to develop fevers might be related to a deficiency of their immune system to produce adequate amounts of endogenous pyrogen (EP). This was refuted when Kasting et al (1979) demonstrated that leukocytes from both the ewe and the lamb at the time of parturition were able to produce EP and that it did not differ from the EP in non-pregnant ewes.

These experiments led to the speculation that fever suppression might be due to some circulating substance that increased in concentration in both the mother and the lamb. The substance that best qualified was AVP. Circulating levels of AVP increased approximately four days prior to birth and returned to normal approximately 32 hours after parturition (Kasting, 1978).

Experiments in near term guinea pigs also revealed an inability to develop normal fever in response to endotoxin. Again, the suppression of fever was seen in near partuition females as well as the newborns. Immunohistochemical experiments revealed that AVP containing neurons showed increased staining content in septal and amygdaloid projections around parturition. The changes lasted until one day post-partum after which normal AVP staining returned (Merker et al, 1980).

AVP perfused into the ventral septal area (VSA), or into the lateral cerebral ventricles results in an abolished or diminished febrile response to endotoxin challenge (Cooper et al, 1979, Kovaks & De Weid, 1983). Stimulation of endogenous AVP release into the VSA suppresses PGE_1-induced fever, and a V_1 antagonist to AVP injected into the VSA diminishes the antipyretic effect of AVP during PGE_1 or endotoxin induced fever (Naylor et al, 1988, Kasting & Wilkinson, 1986). The accumulated evidence strongly implicates centrally-released AVP as an endogenous antipyretic.

E. Peripherally Released AVP

The question remains, however, as to the function or possible role of the large release of AVP into the peripheral circulation during endotoxin or live bacterial challenge (Kruk & Sadowski, 1978).

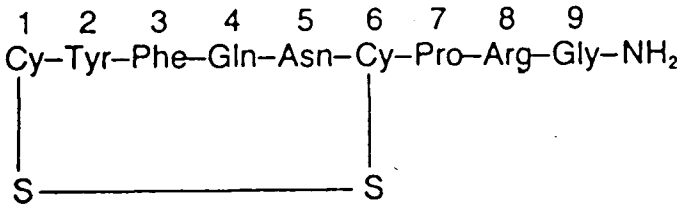
AVP mediates peripheral effects through two separate receptor systems: the V_1 receptors located on vascular smooth muscle and hepatocytes which are responsible for the pressor and glucogenolytic effects and the V_2 receptor subtype which is largely responsible for the antidiuretic actions of the kidney. The V_1 receptor works through activation of phosphatidyl inositol (PIP_2) and intracellular Ca^{++} changes. The V_2 receptor type found in renal tubules exerts its action through the adenylate cyclase second messenger system. Manning and associates (1981) have developed relatively specific antagonists to these two receptor types. There is a third receptor type that mediates the CRF activity of vasopressin which appears to differ from both the V_1 and V_2 receptor types (Jard et al, 1986).

Figure 1 shows the structure of AVP and the V_1 receptor antagonist (M-AVP) utilized in this study.

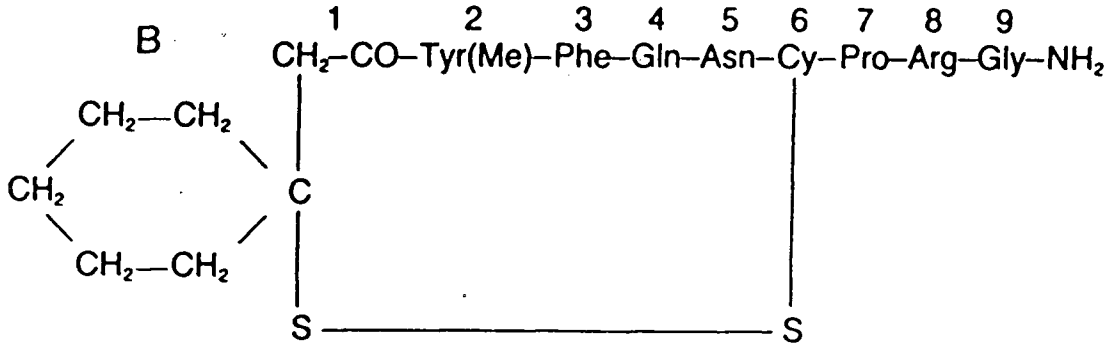
Figure 1

Structure of AVP and V_1 receptor antagonist d(CH_2)$_5$-Tyr(Me)AVP (M-AVP).

A



B



d$(CH_2)_5$Tyr(Me)AVP

AVP's primary known effect is that on water balance. AVP acts on the collecting ducts and distal tubules of the kidneys to resorb water maintaining fluid balance in the face of dehydration. This action is mediated primarily by V_2 receptors and is activated by osmoreceptors.

AVP is also well recognized for it's pressor effects. It decreases heart rate and increases blood pressure when infused (Malayan et al, 1980). In vitro, AVP is a potent stimulator of gluconeogenesis in the liver and concentrations required for this are well within physiological concentrations seen during infection in vivo (Malayan et al, 1980, Hems & Whitton, 1980).

The reticulo-endothelial system (RES) and particularly the phagocytic activity of the cells are directly involved in host defense to bacterial invasion. AVP has important implications in the maintenance of phagocytic integrity during circulatory shock associated with bacterial infections. Brattleboro rats born with a genetic deficiency in AVP, have greatly decreased survival rates during circulatory shock (Altura, 1977). Specifically, AVP has been shown to enhance RES function (Fernandez-Repollet et al, 1983).

An increase in lymphocyte and bone marrow proliferation can be stimulated by AVP (Whitfield et al, 1970, Perris & Hunt, 1988). Recent evidence has indicated that AVP may have a direct effect on the immune system by replacing the IL-2 requirement for gamma interferon production (Johnson & Farrar, 1982).

AVP has the ability to act as a corticotropin releasing factor (Clayton et al, 1965). During infection, both corticosteroids and AVP increase and AVP may be a significant contributor to ACTH release from the anterior pituitary. Recent evidence suggests that AVP may also act directly on the adrenal cortex to synergize with the effect of ACTH in releasing cortisol (Brooks & Blakemore, 1988).

F. Rationale for Investigation

In light of the fact that AVP is released during fever and the plethora of physiological effects AVP is known to have, the following experiments were conducted to determine if peripherally released AVP has a beneficial effect on host defense and recovery from infection due to live bacteria. This also included the development of an appropriate model of live infection from which these data could be collected.

1. Animal Model

As previous experiments testing the effects of AVP during fever had utilized primarily endotoxin recovered from the inside wall of gram-negative, heat-killed bacteria, the present model was developed to investigate the role of AVP during a live infection, thus more closely simulating physiological conditions. AVP was measured to confirm the release into the plasma during live infection.

2. Series I- AVP Infusion during infection

During endotoxin and live bacterial challenge vasopressin is secreted at levels far above those required to exert its antidiuretic effects. This study was conducted to augment the endogenous AVP release in order to amplify any physiological response as well. AVP was infused for one hour prior to injection of the bacteria and the infusion continued for six hours.

3. Series II- Infusion of AVP V_1 Antagonist during infection

The effects of the V_2 receptors are fairly well established, and are mainly involved with water balance. The physiological functions of the V_1 receptor type appear to closely contribute to homeostasis during illness, thus the V_1 antagonist was infused

following a similar protocol as with AVP to elucidate the possible role AVP acting at the V_1 receptor might serve during infection.

4. Series III- AVP Antagonist infusion with repeated bacterial injections

The experiments reported here were carried out to look at the role of AVP in a long-term infection. The V_1 antagonist was infused for six hours per day for a series of three days, bacteria were injected twice daily for the same three days to insure that the infection was maintained.

METHODS

A. Growth of Bacteria

1. Preparation of growth medium

Brain Heart Infusion (BHI) broth (Difco Laboratories) was utilized for all experiments. BHI broth was prepared by mixing to ten times strength in distilled water, heating while stirring to help dissolve. This solution was then autoclaved for 20 minutes to ensure that no bacteria remained viable. The cooled broth was then placed in dialysis tubing, tied at each end, and placed into a large pyrogen free water bath (containing no endotoxin or other substances known to cause fever) ten times original volume. The bath remained in a 4 ^{o}C cold room for 42 hours. This process insured that all endotoxin and other bacterial products remained inside the dialysis tubing. The solution outside the dialysis tubing was then transferred to autoclavable containers and autoclaved for 20 minutes, which then becomes the medium.

2. Preparation of agar plates

A 40g/liter solution of Tryptic Soy Agar (B.B.L. Microbiology Systems) was prepared by heating and autoclaved for 20 minutes. The solution was then placed in a 40 ^{o}C water bath and cooled to 45-50 ^{o}C. When cool, the agar was poured out into plastic agar plates using aseptic techniques to just cover the bottom of the plates. The plates were allowed to cool upright at air temperature for 24 hours then stored upside down in air-tight plastic bags in a cool place. MacKonkey Agar plates, which inhibit growth of gram positive organisms, were made as above except using MacKonkey Agar

medium (Difco Laboratories). Blood Agar plates were made by adding sterile sheep blood to the Tryptic Soy Agar to make a 2% solution after the agar had cooled to 50 °C.

3. Preparation of Bacterial plate

Bacteria were obtained frozen from Bacteria America Type Culture Collection, Maryland. The serotype was 026B6 (E. coli). This bacterial strain is the source of the endotoxin which was used in the previous experiments done in this laboratory. This served to make comparisons more valid. The bacteria were stored at -70 °C. A small metal loop was dipped into the bacterial suspension in the vial and spread onto a blood agar plate to begin the culture. Each time a loop of bacteria was taken for a seed culture a new plate was made and the integrity of the new colony ensured by gram staining or using MacKonkey agar plates, which determines whether the organism is gram - or gram +. In addition, an API test strip (Analytab Products Division Sherwood Medical) which determines the exact biochemical profile of the bacteria was done periodically.

4. Preparation of Bacteria for injection

A seed culture was made by taking a single isolated colony from the bacterial plate and suspending it in 10 ml. of sterile broth. The solution was incubated at 37 °C and allowed to grow to stationary phase overnight. This seed culture was used to inoculate the B.H.I. broth at a concentration of 0.1 ml per 100 ml. The solution was incubated at 37 °C. Inoculation was considered Time 0.

5. Growth Curve

Turbidity was used as an index of the concentration of the bacteria at various times, and was assessed by using a Bausch and Lomb Spectronic 20 at 560 nm wavelength. Samples were taken every 30 minutes from the bacterial culture and read

against a blank of sterile B.H.I. broth. Each time a sample was taken a 0.1 ml sample was also taken and a 1:10 serial dilution performed eight times. Each dilution was then plated by placing 0.1 ml on a sterile agar plate and then spread with an alcohol and flame sterilized glass rod bent into an L shape. The plates were incubated for 24 hours. The bacteria form discreet colonies (Colony Forming Units) which can then be counted. Concentration of bacteria for each time period was determined by taking an average of countable plates.

Colony Forming Units (CFU's) were plotted against optical density and also against time so that absorbance could be used to determine time of harvest for a certain bacterial concentration. Figure 2 shows the graph of absorbance vs CFU's and Time vs CFU's.

B. Surgical Preparation

1. Construction of surgical materials

U-tubes: A 20-g needle was bent around a cylindrical metal object placed in a vise to form a uniform U shaped curve in the middle of the needle. Both ends were scored and cut off. The ends were then made smooth by sanding.

Plugs: Paper clips were cut in approximately 1 mm lengths then smoothed at one end.

I.V. tubing: Silastic tubing (.025 in. ID xA .047 in. OD, Dow Corning) was cut in 110 mm lengths with a bevel at one end. A small cuff made out of PE 190 was slipped over the beveled end and positioned 40 mm from the tip.

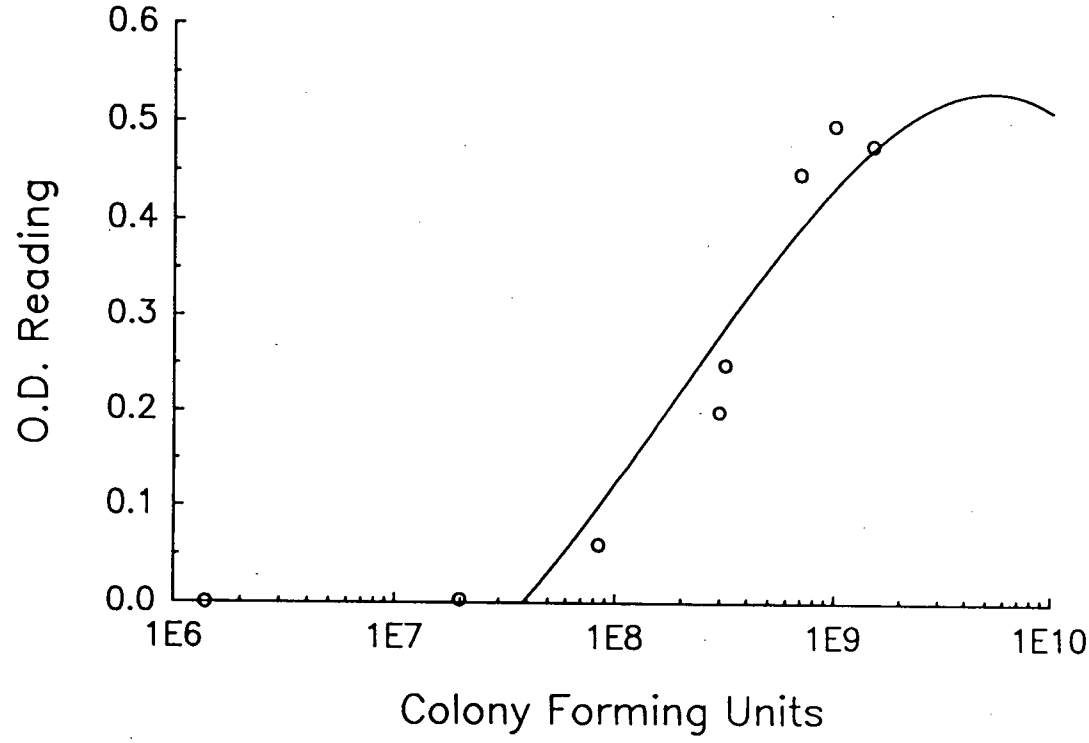
2. Surgical procedure

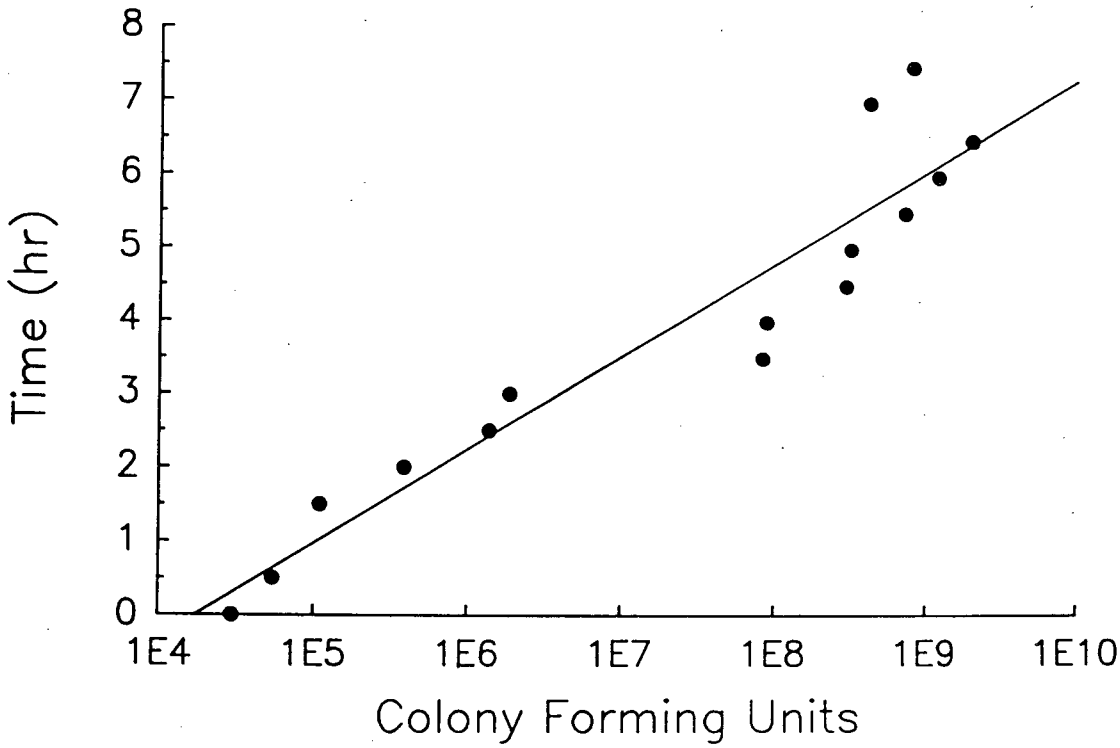
Male Sprague Dawley rats (275-300g) were surgically prepared under pentobarbital anaesthesia (0.1ml/kg, intraperitoneal (i.p.) Before all surgery the rats were pinched on the foot to insure adequate anaesthesia, the chest, side and top of head were shaved and swabbed with 70% alcohol. A longitudinal incision in the skin was made directly above the right carotid artery approximately 1 cm long. The jugular vein was exposed and two loose knots approximately 1 mm apart were made around the vein. Using very fine scissors a small V shaped cut was made between the two knots and the proximal knot was quickly tied off to prevent bleeding.

Figure 2

A) Absorbance reading vs. average number of colony forming units (cfu's) per ml.

B) Time vs. cfu's/ml.


0.6
0.5
0.4
0.3
0.2
0.1
0.0
O.D. Reading
1E6
1E7
1E8
1E9
1E10
Colony Forming Units


8
7
6
5
4
3
2
1
0
Time (hr)
1E4
1E5
1E6
1E7
1E8
1E9
1E10
Colony Forming Units

The cannula was attached to a 1 ml syringe and filled with heparinized saline (40 ul/ml.). The tip was inserted into the vein and advanced until it was against the wall of the right atrium, as seen by pulsations caused by ventricular contraction. The catheter was then pulled back just enough so that pulsations were no longer visible. The cuff of the catheter was repositioned to rest against the tissue and tied off securely on each side. A few drops of blood were then withdrawn to test for patency in various positions. The line was filled with heparin and plugged.

A small incision was made at the top of the skull and a large bore needle pushed under the skin behind the ears to exit at the neck. The catheter was fed through the needle and pulled through until it came out on the head.

The rat was placed in a stereotaxic device, an incision made forward from the exit of the catheter along the saggital suture approximately 20 mm long. The fascia attached to the skull was scraped away and five holes drilled into the skull. Five stainless steel jeweler's screws were placed into the holes. A piece of PE 100 approximately 3 cm long was filled with saline and then attached to a U-tube. The U-tube was then attached to the Silastic tubing exiting at the neck. The U-tube was attached to the skull with dental acrylic which flowed around the U-tube and screws.

The rat was then removed from the stereotaxic device and a radiotelemetric transmitter (Minimeter Inc.) designed for use in rats was placed in the peritoneal cavity which allowed constant monitoring of body temperature.

Rats were allowed to recover until their normal circadian body temperature rhythm resumed (about 1 week). Food and water were given ad libitum.

The day before the experiments were to begin the rats were placed in a wire mesh cage containing a spring and ball bearing assembly. The jugular catheter was connected to a 1 ml syringe outside the cage via PE 100 tubing for blood sampling and

infusions. The cage was enclosed in an isolation box with a 12:12 light-dark cycle and fan for circulation. All experiments were performed at an ambient temperature of 24 $\pm$ 1 ^{o}C. All experiments were begun at 11:00 $\pm$ 1 hour.

C. AVP Assay

AVP plasma concentrations were measured using a radioimmunoassay with a sensitivity of 0.13-0.22 pg/tube. Rabbit anti-AVP antiserum was used at a final concentration of 1:810 000. The assay used 50 µl of anti-AVP antiserum, 50 µL of sample, 250 µL of buffer (0.1M sodium phosphate, pH 7.2 containing 0.01M EDTA, 0.05 M NaCl, 0.02% sodium azide, and 0.01% BSA) and 100 µL of ^{125}I-AVP (New England Nuclear) at 4000 counts per minute (cpm) per tube. The assay was incubated at 4 ^{o}C for 1 day without label and then 2 days with label. The double antibody technique was used to precipitate the bound label and the pellet was counted in a gamma spectrometer and amount of bound AVP calculated by log-logit method. Intraassay variability was 8.9% and interassay variability was 8.8% for a 1.04 pg standard. The 50% bound to unbound ratio (the ratio of labelled peptide bound in the presence of AVP standard to that bound in the absence of any AVP) was 1.72 and the slope of the curve was -1.72. The antibody cross-reacts 0.2% with OXY and less than 0.01% with all other peptides tested; bombesin, cholecystokinin, bradykinin, angiotensin II, neurotensin, leu-enkephalin, thyroid stimulating hormone releasing hormone and vasoactive intestinal polypeptide.

D. Development of Model

1. Dose-Response Curve

Twelve rats were divided into three groups (N=4 in each group) and a different concentration of bacteria administered to each group. The low dose was 10^6 cfu/ml, the medium dose was 10^7 cfu/ml, and the high dose was 10^8 cfu/ml. A volume of 0.5 ml of live bacteria in BHI was injected i.p. to each group. Rectal temperatures were taken at time 0, 0.5, 1, 2, then every 2 hours for 2 days ending the first day at 22:00 and beginning again on Day 2 at 08:00. Time was plotted against temperature to determine the dose-response.

2. Pilot experiments

The highest dose during the growth phase was chosen which was approximately 3 x 10^9 cfu/ml (.38 O.D.). This was then concentrated to maximize the amount of live bacteria. In 50% of the rats a 1 ml injection was given directly into the lungs using an endotracheal tube. The other rats received 0.5 ml of the same concentration administered i.v. A concentration of bacteria harvested at 0.25 A_{560nm} was decided upon because it was well within the growth phase and could be concentrated in order to more precisely determine the amount of live bacteria being delivered. The bacteria was concentrated x 5 to achieve a final concentration of 1.5 x 10^9 cfu/ml. This dose was administered as a single injection of 0.5 ml into the intraperitoneal cavity.

Baseline body temperature was taken for the three days prior to injection and an average calculated for the group. Plasma AVP levels were determined by RIA. Plasma glucose and plasma osmolality were also determined using a Beckman glucose analyzer and osmometer.

E. Experimental series I

AVP infusion with i.p. injection

Male Sprague Dawley rats (275-300g) were divided into two groups (n=9 in each group). They were surgically prepared with chronic indwelling radiotelemetric transmitters as previously described. Rats were housed individually with a 12:12 light dark cycle and given food and water freely.

Baseline body temperature for each hour was recorded for 3 days prior to injection. Change in body temperature was taken for that time of day taking circadian differences into account. For example a temperature recorded at 11:00 would be compared with baseline body temperature at 11:00 averaged from 3 control days.

Rats were injected with 0.5 ml live E. coli bacteria i.p. at a concentration of 7 x 10^8 cfu/ml concentrated x 5. One group received a saline infusion one hour prior to injection and continued for 6 hours and the other AVP (1.75 ng/hr) by a similar i.v. infusion.

Body temperature changes from baseline were noted at 6, 12, 24, and 48 hours after injection of the bacteria. Blood samples (O.5 ml) were taken at 16:00 and 08:00 prior to the experiment (control) then each hour for 5 hours until the infusion stopped. The blood was centrifuged immediately, and the plasma aliquoted and frozen. The red cells were resuspended in saline and returned to the rat, directly after sampling (within 15 minutes). For the next two days blood samples were taken twice daily. The plasma was assayed for AVP and glucose as previously described.

F. Experimental series II

Antagonist infusion with i.p. injection

Rats were divided into two groups (N=6 in each group) and surgically prepared with chronic intraatrial catheter and biotelemetry devices as previously described. Rats were allowed to recover for one week prior to injection of bacteria.

Body temperature was monitored for three "control" days prior to the beginning of the experiment. Change in body temperature was taken from baseline at that time of day averaging the three control days.

One group received M-AVP V_1 antagonist (0.5 ml/hr, 3.6 μg/ml) while the other group served as control and received saline infusion. Infusions began 1 hour prior to the injection of bacteria and continued for 6 hours. Injection of 0.5 ml, 3 x 10^8 cfu/ml bacteria was at 11:00 after the three control days.

Blood samples were taken at 16:00 and 08:00 prior to the start of infusion, then 6 consecutive hours after the start of infusion. Samples were taken twice daily for the next 2 days. Samples were assayed for glucose using a Beckman Glucose Analyzer. Body temperature was compared to baseline at 6, 12, 24, and 48 hours.

G. Experimental Series III

Repeated injection with antagonist infusion

Rats were surgically prepared with right jugular catheter and radio transmitters and allowed to recover until circadian rhythm was re-established as previously described. The rats were separated into two groups (N=9 in each group). E. coli was grown to 3 x 10^8 cfu/ml and an intraperitoneal injection given twice daily at 09:00 and 17:00. Three

hundred microliters were given on the first day, 0.4 on the second day, and 0.5 ml on the third day. The V_1 antagonist (0.5 ml/hr, 3.6 μg/ml) or saline was infused beginning one hour before injections each day and continued for six hours. Rats were allowed 3 days to recover from the infection, then sacrificed.

White blood cell counts were done at 16:00 and 08:00 prior to the beginning of infusion, and twice daily during the experimental period. Counting was done with a Spencer Bright-line chamber utilizing the four corner squares.

H. Statistical Analysis

Statistics were done using an unpaired Student's T-test for bar graphs, data was considered significant at $p < 0.05$. An ANOVA two-way analysis of variance was utilized for the line graphs to examine statistical differences within and between groups. Data was considered significant at $p < 0.05$.

RESULTS

A. Development of Model

Dose-Response

The preliminary dose-response experiment was done with a 0.5 ml intraperitoneal (i.p.) injection of bacteria. The highest dose (4 x 10^8 cfu/ml) showed an initial hypothermia before development of fever. The two lower doses (4 x 10^6 and 2 x 10^7 cfu/ml) developed a fever lasting approximately four hours with recovery. The two lower doses chosen did not differ from each other. (Fig. 3).

The bolus lung injection caused immediate death within 4 hours of administration if the highest dose was used and no significant response if a more moderate dose was administered. The i.v. injection of the same concentration caused death in three out of four rats. These two models were therefore rejected.

The highest dose, 4 x 10^8 cfu/ml concentrated x 10, via centrifugation and resuspension in sterile BHI broth, was decided upon because it resulted in a fever lasting from 12-24 hours when injected i.p. Normal circadian rhythm resumed thereafter. Harvesting the bacteria at a lower concentration insured that the bacteria were in a growth phase and therefore viable. The fever was characterized not only by an abnormally high body temperature but also a loss of the normal daytime drop in body temperature (Fig.4). This dose was used in subsequent AVP and antagonist infusion experiments.

Release of AVP into the plasma confirmed earlier observations using only endotoxin and was variable (Kasting, 1987). For example, in four of the six rats sampled a peak occurred at 4 hours with AVP levels returning to normal thereafter. In two of the six rats AVP levels remained elevated for up to 48 hours after injection (Fig. 5)

Since i.p. injection gave a relatively long lasting fever and caused AVP release, this was adopted as the model used for Experimental Series I and II.

Figure 3

Dose response to a 0.5 ml i.p. injection of live E. coli bacteria. The three concentrations used were 4 x 10^6 cfu/ml, 2 x 10^7 cfu/ml, and 4 x 10^8 cfu/ml. Temperature was monitored every two hours after injection for ten hours, then every two hours starting at 08:00 on day two for ten hours.

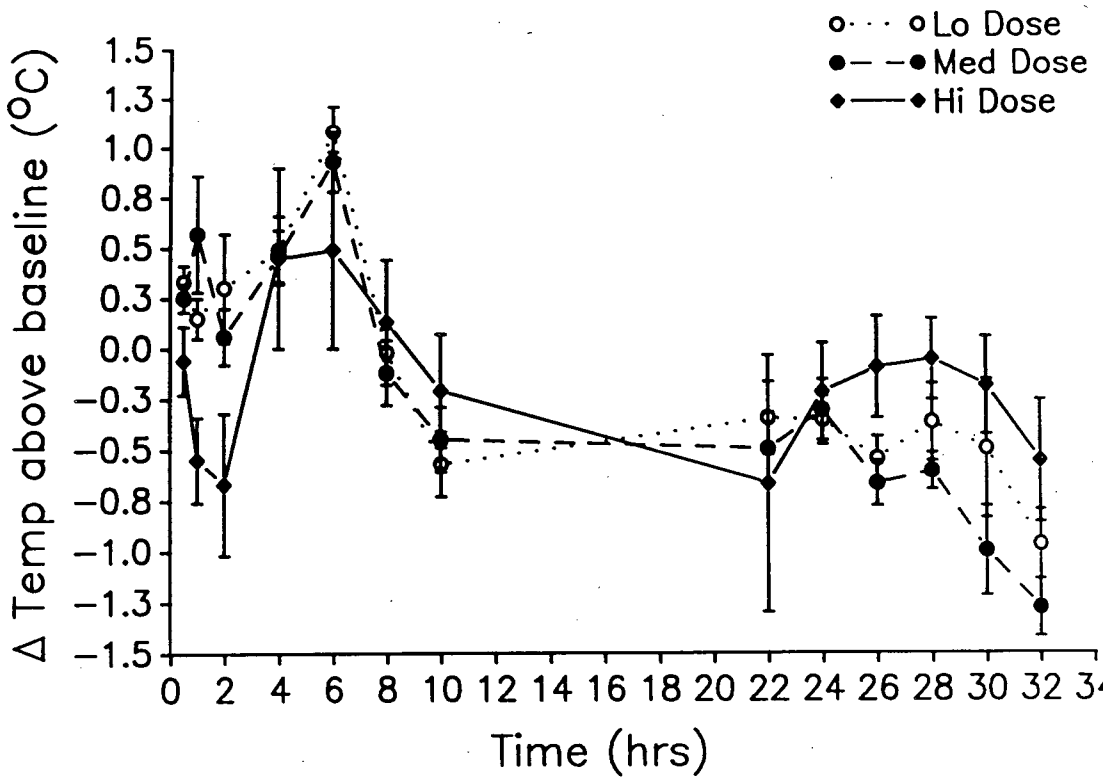

Lo Dose
Med Dose
Hi Dose
Δ Temp above baseline (°C)
1.5
1.3
1.0
0.8
0.5
0.3
0.0
−0.3
−0.5
−0.8
−1.0
−1.3
−1.5
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34
Time (hrs)

Figure 4

Characteristic temperature response to injection of 0.5 ml live E. coli bacteria (3 x 10^8 cfu/ml). Black arrow indicates time of injection. Note that injection of the bacteria abolished the normal daytime drop in body temperature. Vertical lines indicate 24 hour intervals. Averaging interval is 60 minutes.

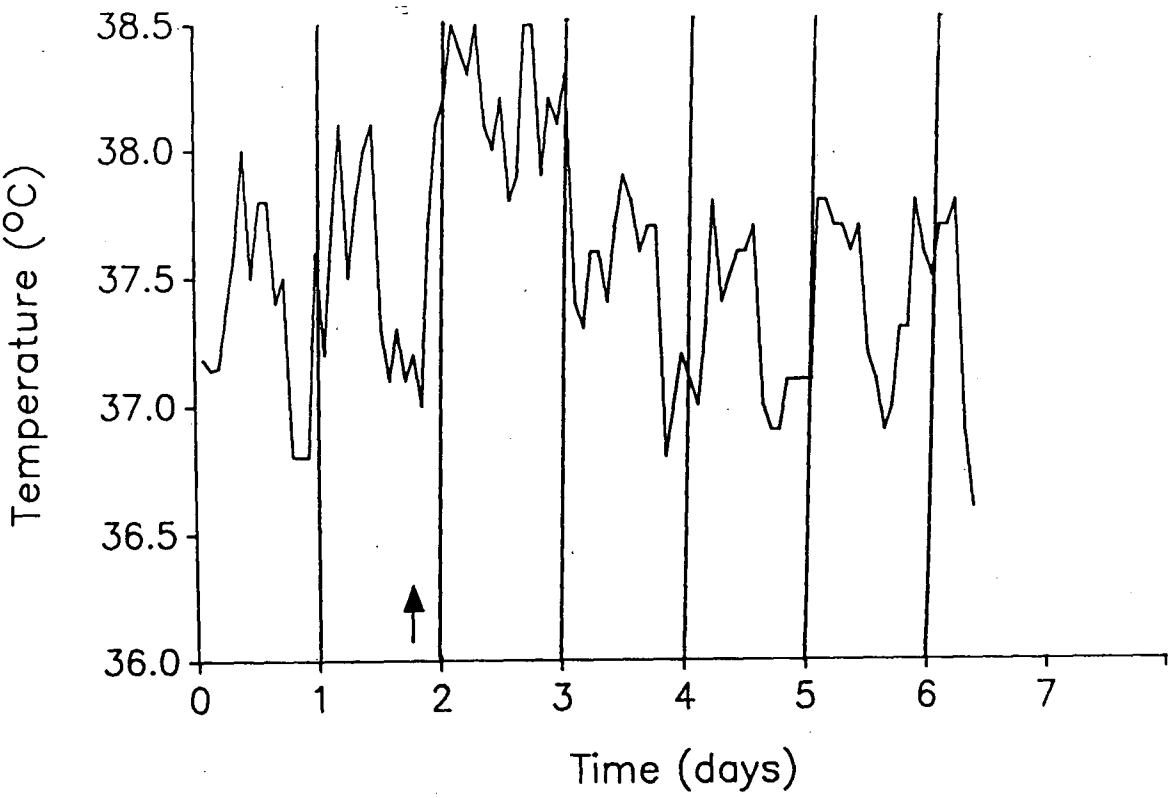
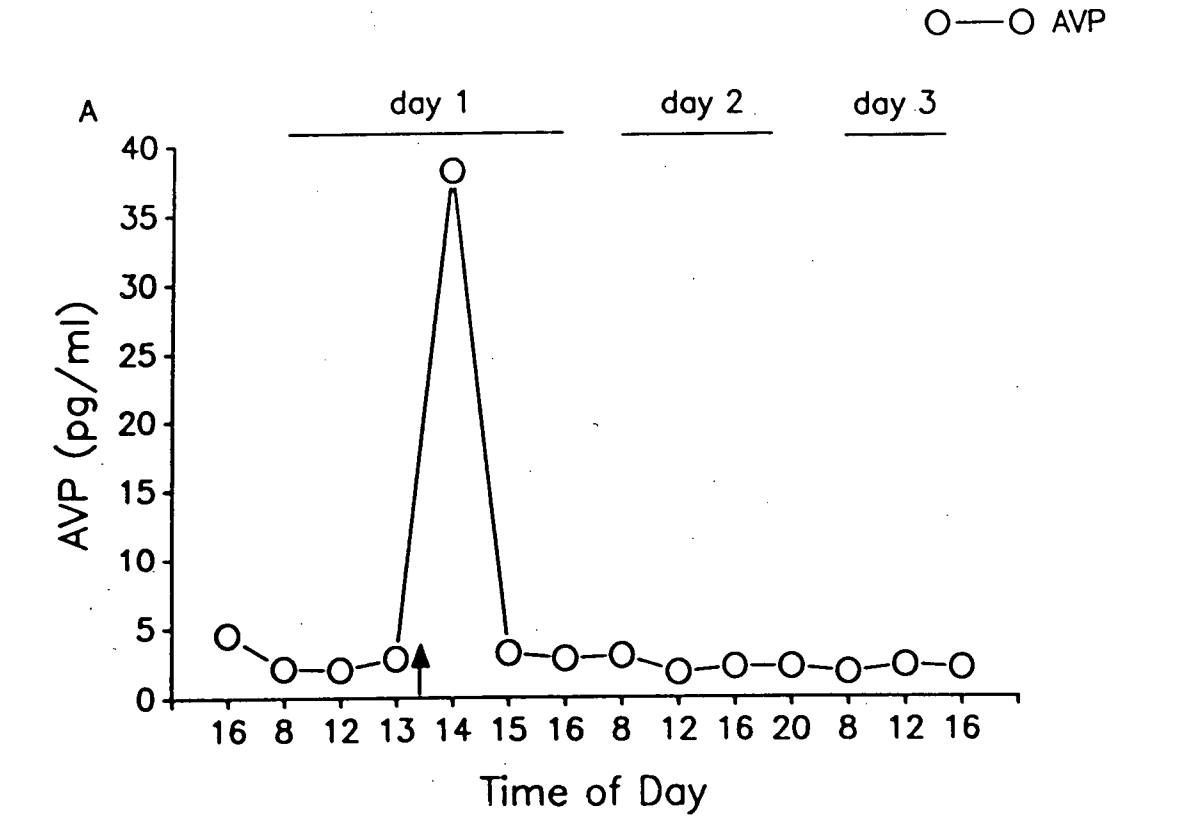
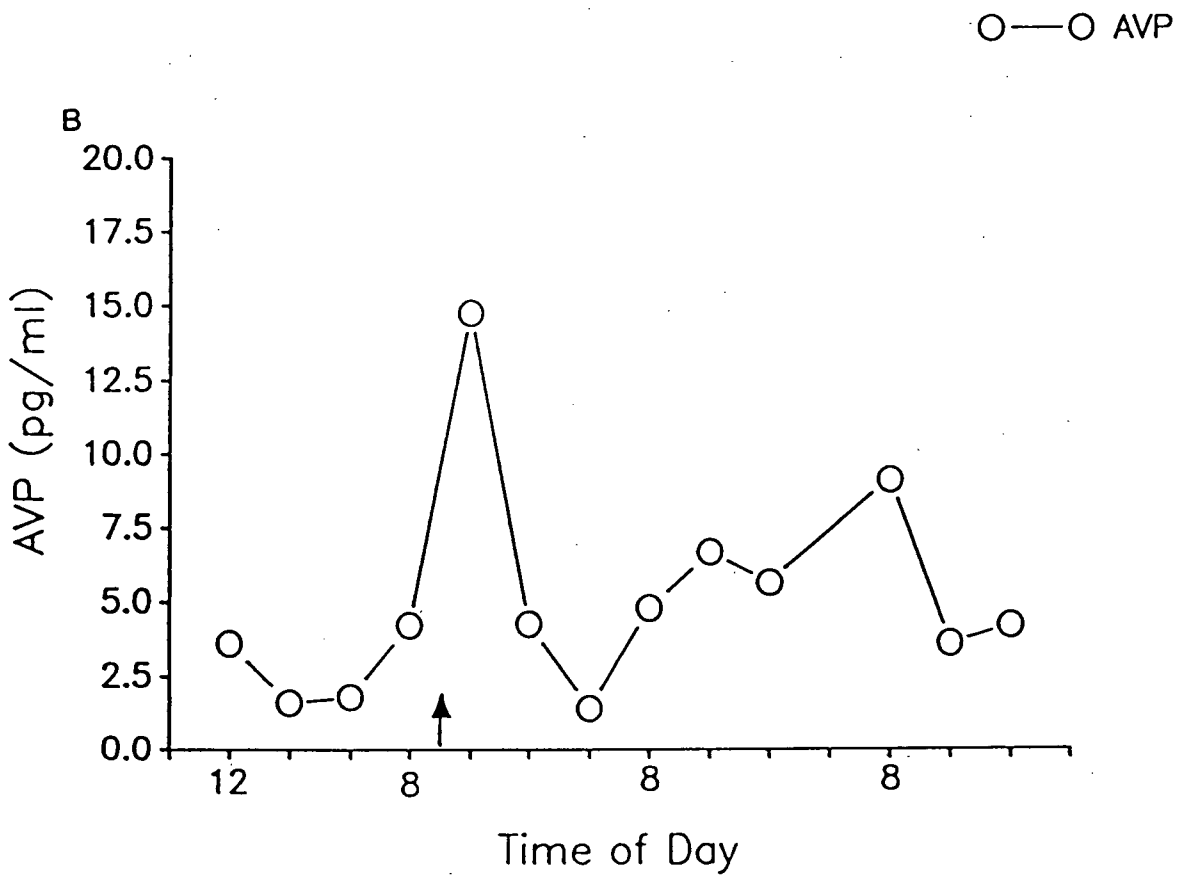

38.5
38.0
37.5
37.0
36.5
36.0
Temperature (°C)
0
1
2
3
4
5
6
7
Time (days)

Figure 5

Demonstration of variable AVP responses to injection of 0.5 ml live E. coli bacteria (i.p.). Black arrow indicates time of injection. a) Peak occurs at four hours. b) AVP levels peak then return to normal and increase again 24 hours later. (N = 1 in each group).


O—O AVP
A
day 1
day 2
day 3
AVP (pg/ml)
40
35
30
25
20
15
10
5
0
16 8 12 13 14 15 16 8 12 16 20 8 12 16
Time of Day


O—O AVP
B
AVP (pg/ml)
20.0
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
12 8 8 8
Time of Day

B. Experimental Series I

AVP Infusion with i.p injection

Baseline AVP levels for the two groups were not different (saline = 3.0 + 0.3 pg/ml, N = 6, AVP = 3.1 $\pm$ 0.3 pg/ml, N = 5). After 1 hour infusion of AVP or saline, AVP levels were significantly higher in the AVP infused group and unchanged in the saline infused group (saline = 3.8 $\pm$ 1.2 pg/ml, AVP= 134.1 $\pm$ 36.6 pg/ml, T-test;p < 0.05). Four hours after E. coli injection AVP levels in both groups were elevated; however, the AVP group was well above the saline group (saline = 43.9 $\pm$ 21.9 pg/ml, AVP =167.5 $\pm$ 37.8 pg/ml, T-test; $p < 0.05$). Fig. 6 shows the mean AVP response in each group over the course of the experiment. Fig. 7 shows the mean AVP response to infusions and injections taken one hour after infusion and four hours after injection,(T-test;$P < 0.05$).

AVP significantly altered the febrile response to the bacteria. Responses to the bacteria were highly variable in the first few hours (Fig. 8). Temperature changes for saline infused rats (N = 12) at 6 hours were 0.38 $\pm$ 0.55 ^{o}C, while those for the AVP infused rats (N = 12) were 0.90 $\pm$ 0.19 ^{o}C. This was not significantly different between groups. The AVP also caused a pronounced hypothermic phase at 12 hours, (-0.61 $\pm$ 0.27 ^{o}C as compared to -0.04 $\pm$ 0.19 ^{o}C) for saline infused rats,(T-test; $p < 0.05$). There was a consistent fever at 24 hours but this was not significantly different between groups, (saline = 0.50 $\pm$ 0.18 ^{o}C, AVP = 0.82 $\pm$ 0.26 ^{o}C, T-test). This further demonstrates the lack of normal decline in daytime body temperature.

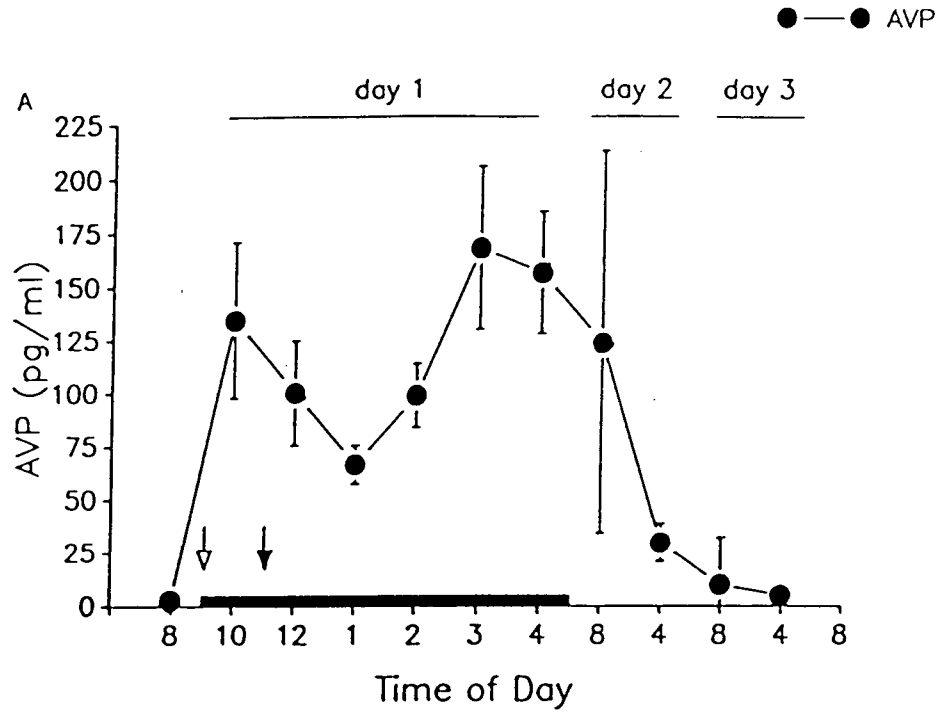
The bacterial dose administered was lethal in 2/9 saline-infused rats and 0/9 AVP-infused rats.

A fever index (indicating both fever height and length) taken for four consecutive twelve hour periods showed a similar trend. There was less variability in the first 12 hours for AVP infused rats. The 12-24 hour fever index showed a hypothermia in the AVP infused rats, although significant only to $p < 0.1$ (T-test). Fever was evident in both groups in the 24 - 36 hour fever index although somewhat less in the AVP infused group (Fig. 9).

Glucose changes were not different between the two groups (ANOVA). The control value for the AVP infused group was 124 ± 5.3 mg/dl and 116 ± 10.6 mg/dl for the saline infused group. Fig. 10 shows the glucose response for the two groups, at no time were glucose levels unusually high in either group. At 5 hours the saline infused group had slightly higher blood glucose levels, although not significantly different, and remained high until the third day after injection. Glucose levels were not different between groups 48 hours after injection (ANOVA).

Figure 6

AVP response to bacterial injection in a) AVP infused (closed circles, N = 9) or b) saline infused (open circles, N = 8). Open arrow indicates start of infusion, closed arrow indicates injection of bacteria. Bar indicates time of infusion. Note difference in scales between a and b.


AVP
A
day 1
day 2
day 3
225
200
175
150
125
100
75
50
25
0
AVP (pg/ml)
8
10
12
1
2
3
4
8
4
8
4
8
Time of Day

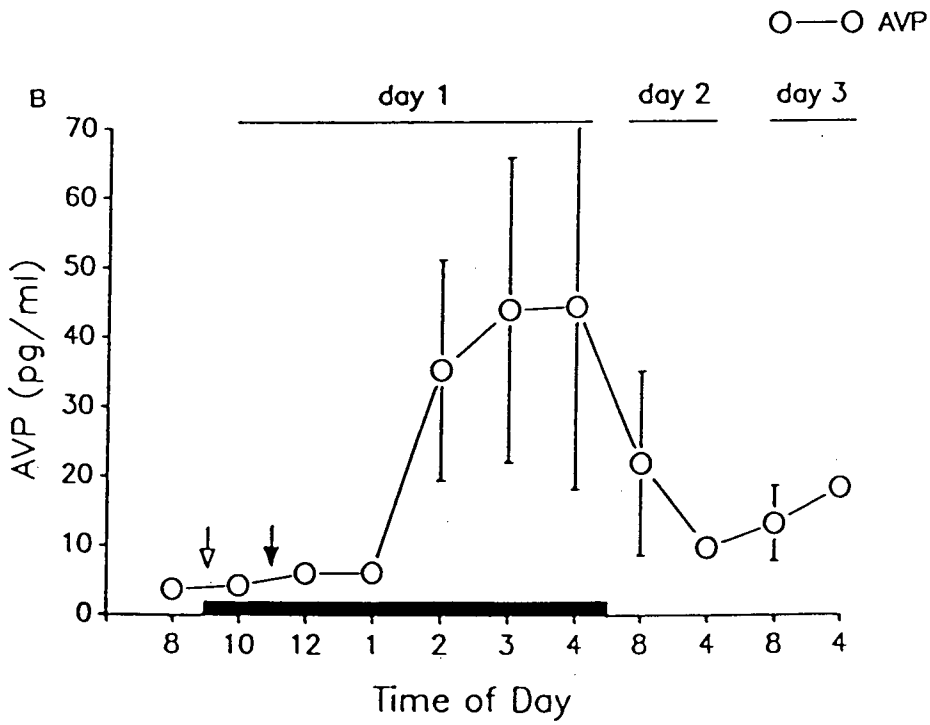

AVP
B
day 1
day 2
day 3
70
60
50
40
30
20
10
0
AVP (pg/ml)
8
10
12
1
2
3
4
8
4
8
4
Time of Day

Figure 7

Average AVP levels in saline (open boxes) or AVP 3.6 μg/ml, 0.5 ml/hr (solid boxes). Infusion began one hour before injection. Samples were taken one hour after infusion and four hours after injection. Open arrow indicates beginning of infusion. Black arrow indicates injection. Unpaired Student's T-test, * $p < 0.05$.

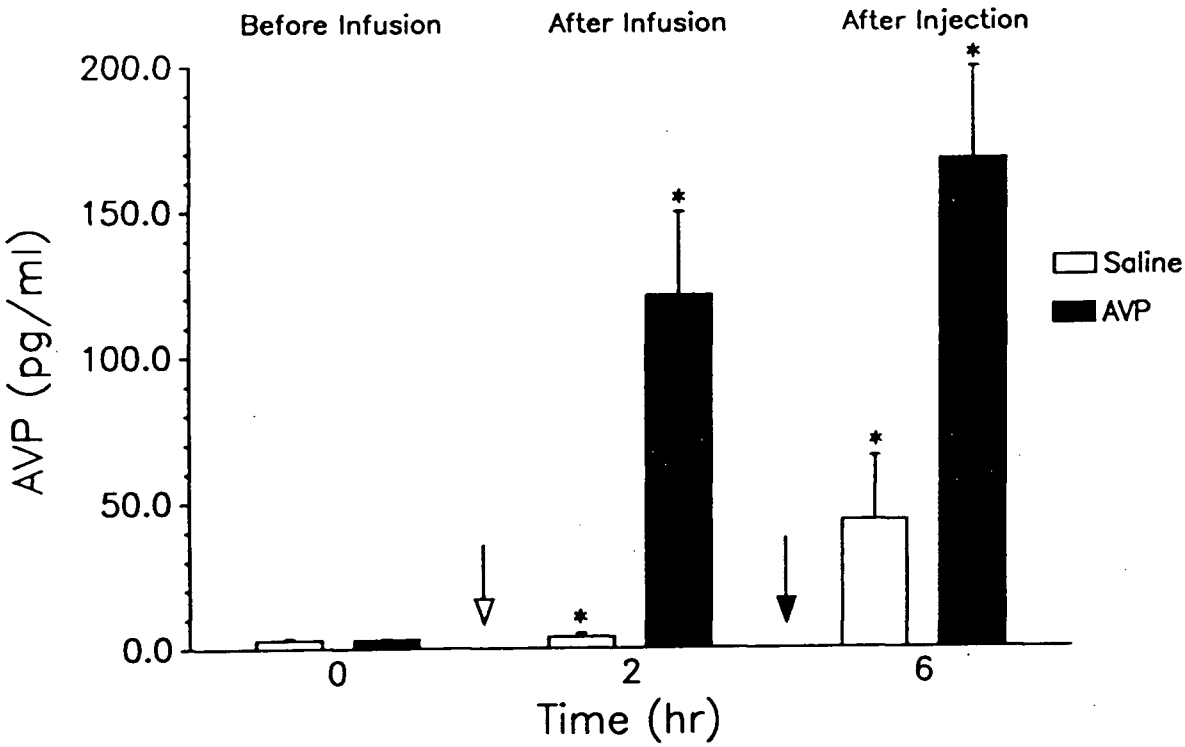

Before Infusion
After Infusion
After Injection
200.0
150.0
100.0
50.0
0.0
AVP (pg/ml)
0
2
6
Time (hr)
Saline
AVP

Figure 8

Temperature response to infusion of saline (open boxes) or AVP (solid boxes) one hour prior to injection of 0.5 ml, 3 x 10^8 cfu/ml bacteria. Comparisons were made at 6 12 24 and 48 hours after injection. Unpaired Student's T-test, * $p < 0.05$.

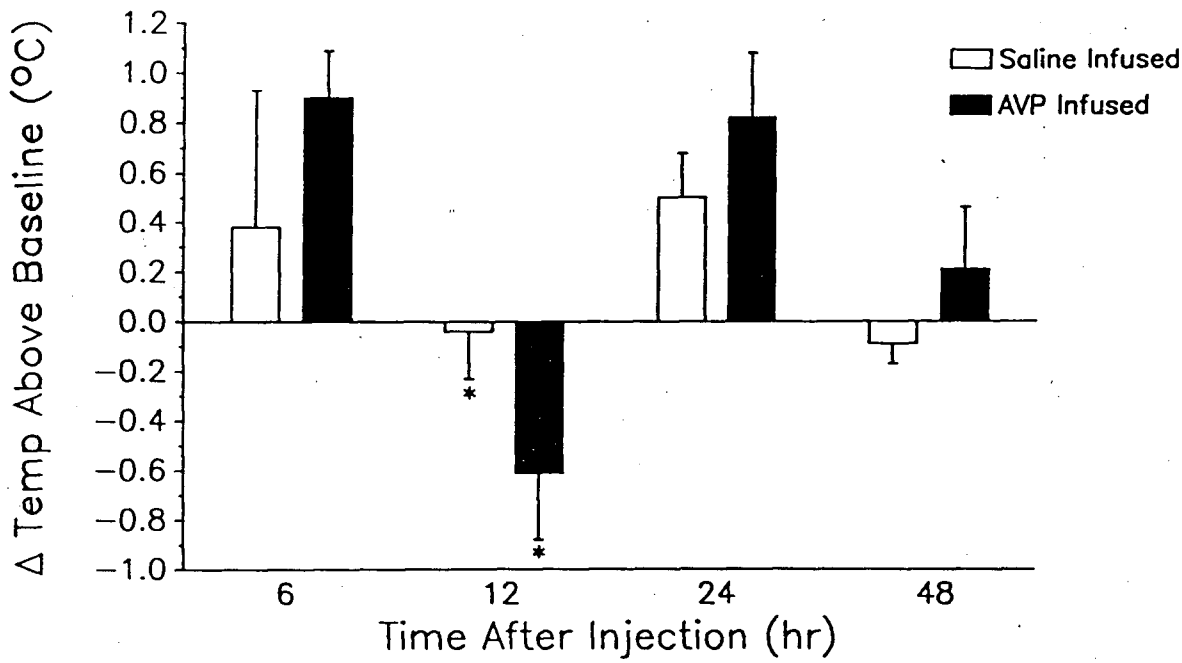

Δ Temp Above Baseline (°C)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
−0.2
−0.4
−0.6
−0.8
−1.0
6
12
24
48
Time After Injection (hr)
Saline Infused
AVP Infused
*
*

Figure 9

Twelve hour fever index. Fever index = ^{o}C hour, so that a one hour fever index of 1 means that the temperature was 1 ^{o}C above baseline for that time of day for a period of one hour, thus taking both fever height and length into account. Differences between the two groups were not significant to $p < 0.05$, ** $p < 0.1$.

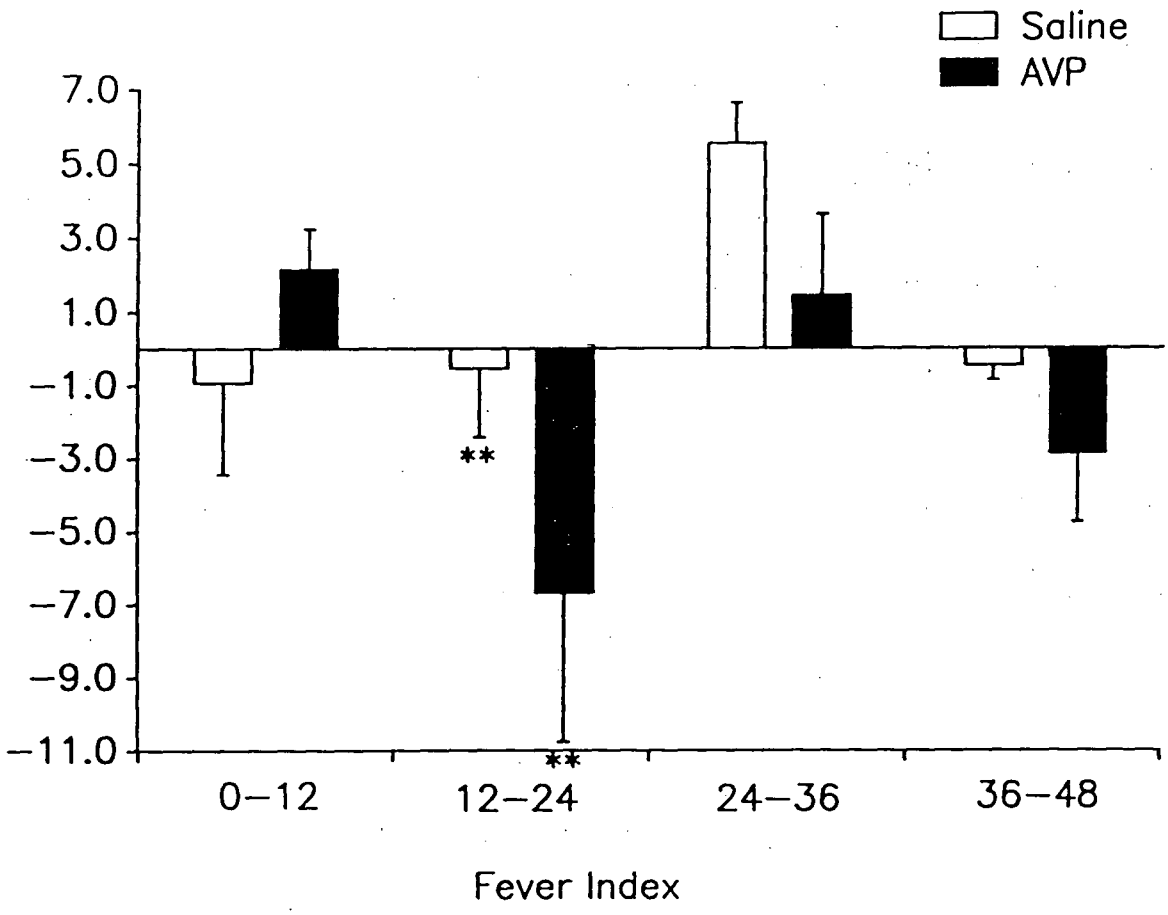
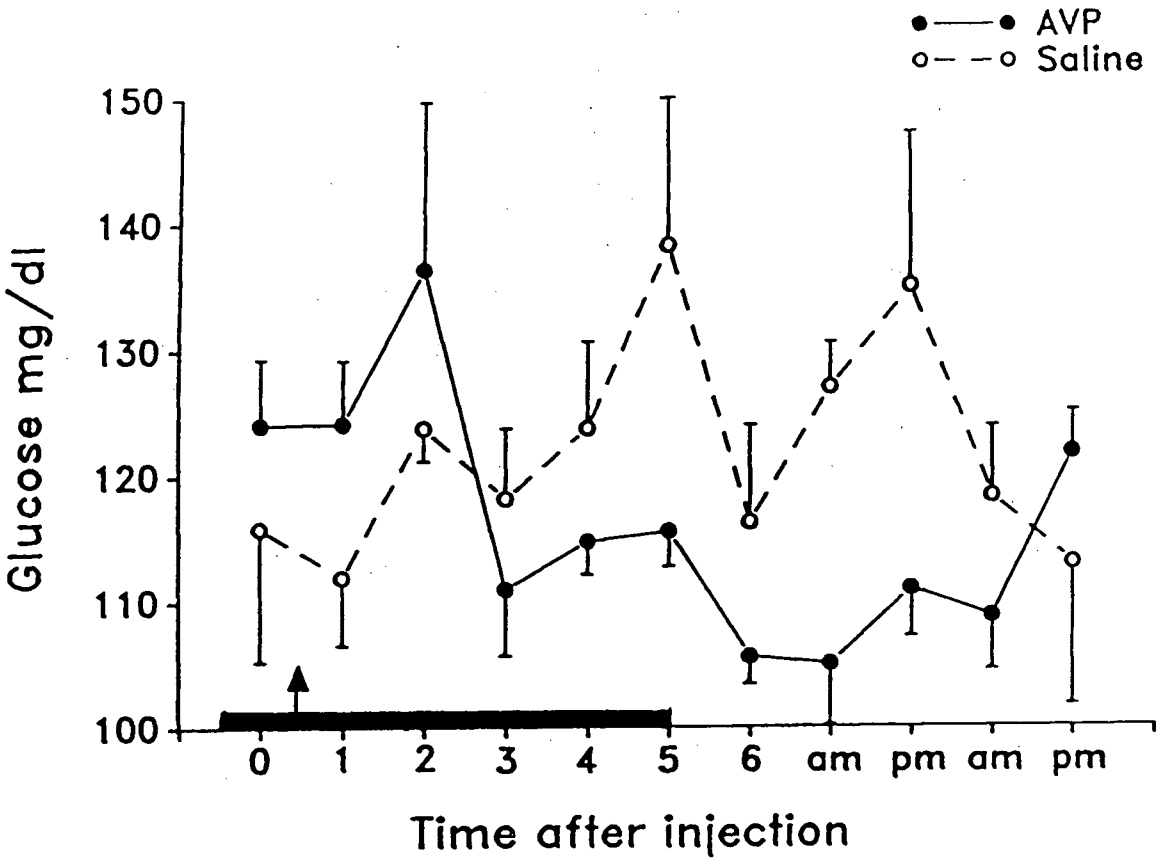

Saline
AVP
7.0
5.0
3.0
1.0
−1.0
−3.0
−5.0
−7.0
−9.0
−11.0
**
**
0−12
12−24
24−36
36−48
Fever Index

Figure 10

Glucose changes after infusion of AVP (closed circles, N = 6) or saline (open circles, N = 6) and injection of bacteria (arrow). Black bar indicates duration of infusion. Differences between groups were not statistically significant. ANOVA two-way analysis of variance. $F = 0.02$, $p > 1.0$.


AVP
Saline
150
140
130
120
110
100
Glucose mg/dl
0
1
2
3
4
5
6
am
pm
am
pm
Time after injection

C. Experimental Series II

Antagonist infusion with i.p. injection

Baseline body temperatures were not significantly different between groups (T-test). Temperature changes from baseline taken at 6 or 12 hours post-injection were not different (T-test): at 6 hours saline = 0.33 $\pm$ 0.11, antagonist= 0.18 $\pm$ 0.26, at 12 hours saline = -0.12 $\pm$ 0.15, antagonist = -0.08 $\pm$ 0.04. The antagonist infused group showed a much higher fever at 24 hours (18 hours after the infusion was terminated) then did the saline infused group (antagonist= 0.39 $\pm$ 0.08, saline = 0.00 $\pm$ 0.05, T-test; $p < 0.05$). By 48 hours both groups had returned to normal except one rat in the saline infused group as seen by the large standard error (Fig.11).

Figures 12 a) & 12 b) show a representative response in each group receiving the same i.p. injection of bacteria. The saline infused rats demonstrated a typical hypothermia followed by loss of daytime drop in body temperature the following day. This temperature response is virtually indistinguishable from the response seen without infusion of either saline or antagonist. The antagonist infused group of rats did not normally exhibit an initial hypothermia. One hour after injection saline infused rats were 0.89 ^{o}C lower than baseline while the antagonist infused rats were only 0.22 ^{o}C lower than normal, T-test;$p < 0.05$.

Blood glucose changes were not significantly different between the two groups (Fig. 13). Control values were 132.0 $\pm$ 5.7 mg/dl saline (N = 2) and 146.0 $\pm$ 0.0 mg/dl antagonist (N = 2). After 1 hour of infusion they were virtually identical 133.5 $\pm$ 4.6 saline and 135.5 $\pm$ 8.4 antagonist. One hour after injection the glucose levels rose in the saline infused group and remained elevated until 3 hours after injection. The antagonist group's glucose levels rose to 157.0 $\pm$ 7.4 mg/dl then dropped to 106.0 $\pm$ 4.9 mg/dl 3 hours after injection. Blood glucose changes were not significant (ANOVA).

Figure 11

Change in temperature from baseline in antagonist infused rats (hatched bar, N = 10) or saline infused (open bar, N = 8) 6, 12, 24, and 48 hours after injection of bacteria. Unpaired Student's T-test, * $p < 0.05$.

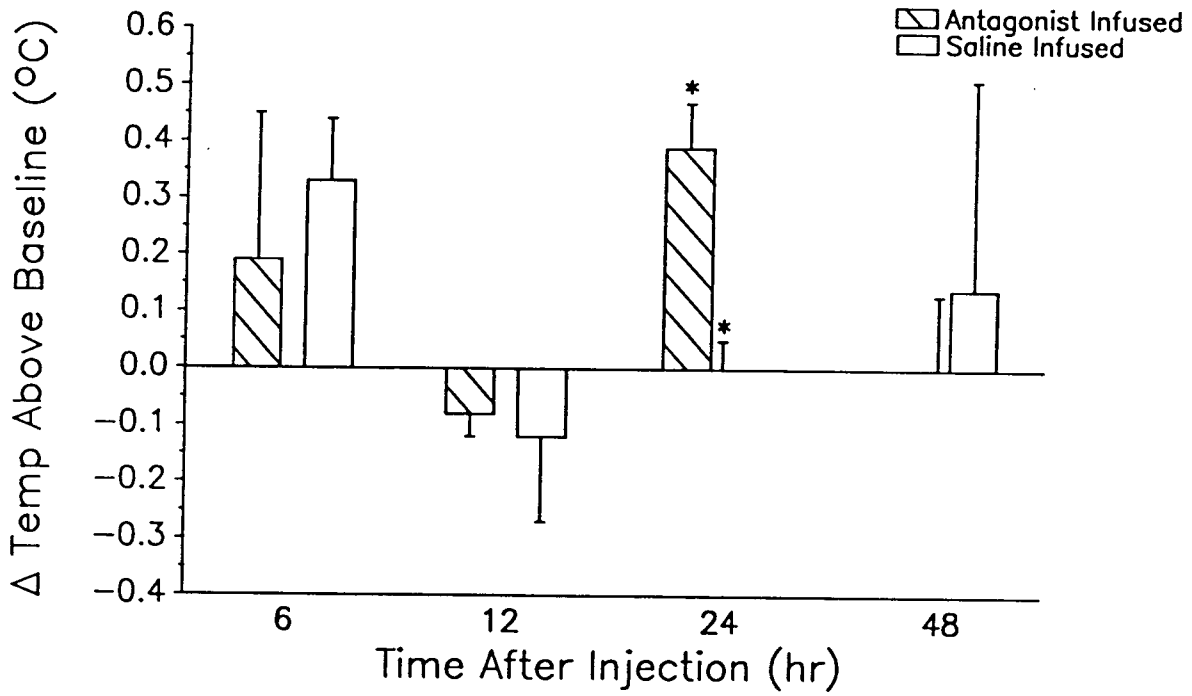
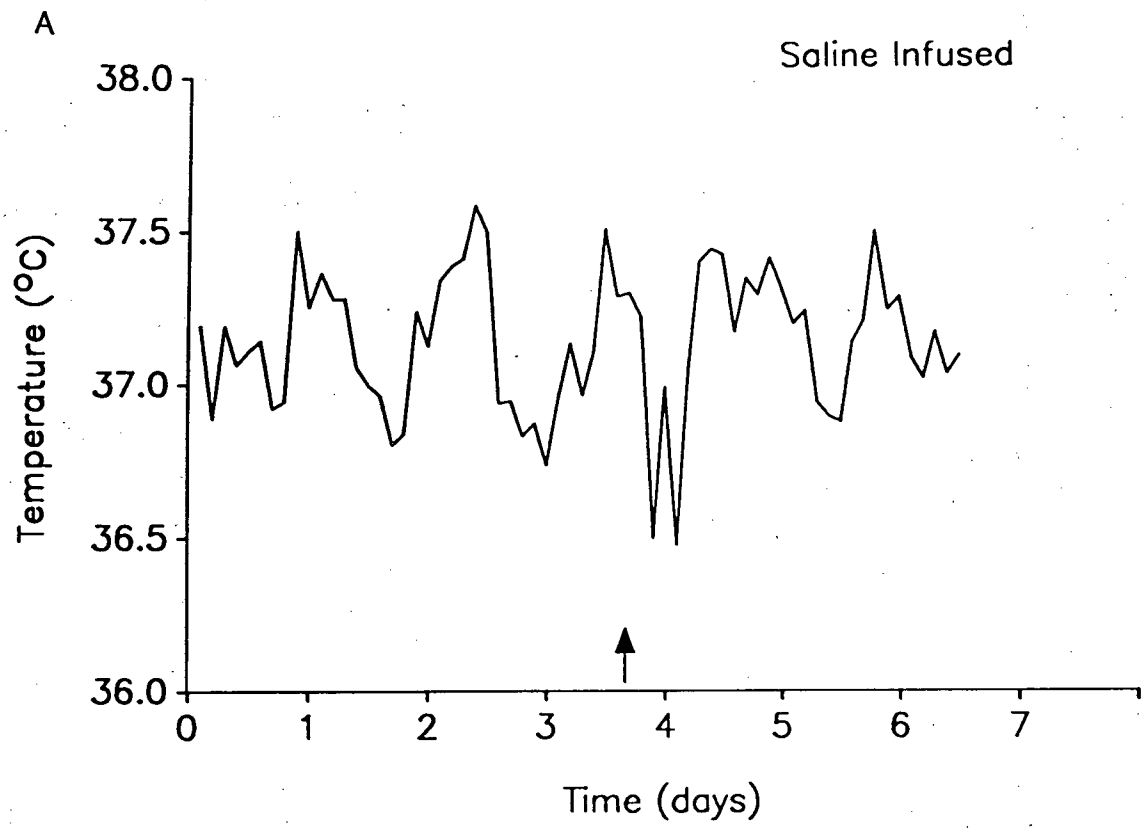

Antagonist Infused
Saline Infused
Δ Temp Above Baseline (°C)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
−0.1
−0.2
−0.3
−0.4
*
*
6
12
24
48
Time After Injection (hr)

Figure 12

Temperature response to injection of bacteria and saline infusion (12 a) or antagonist infusion (12 b). Note difference in temperature scale.


A
Saline Infused
38.0
37.5
37.0
36.5
36.0
Temperature (°C)
0
1
2
3
4
5
6
7
Time (days)

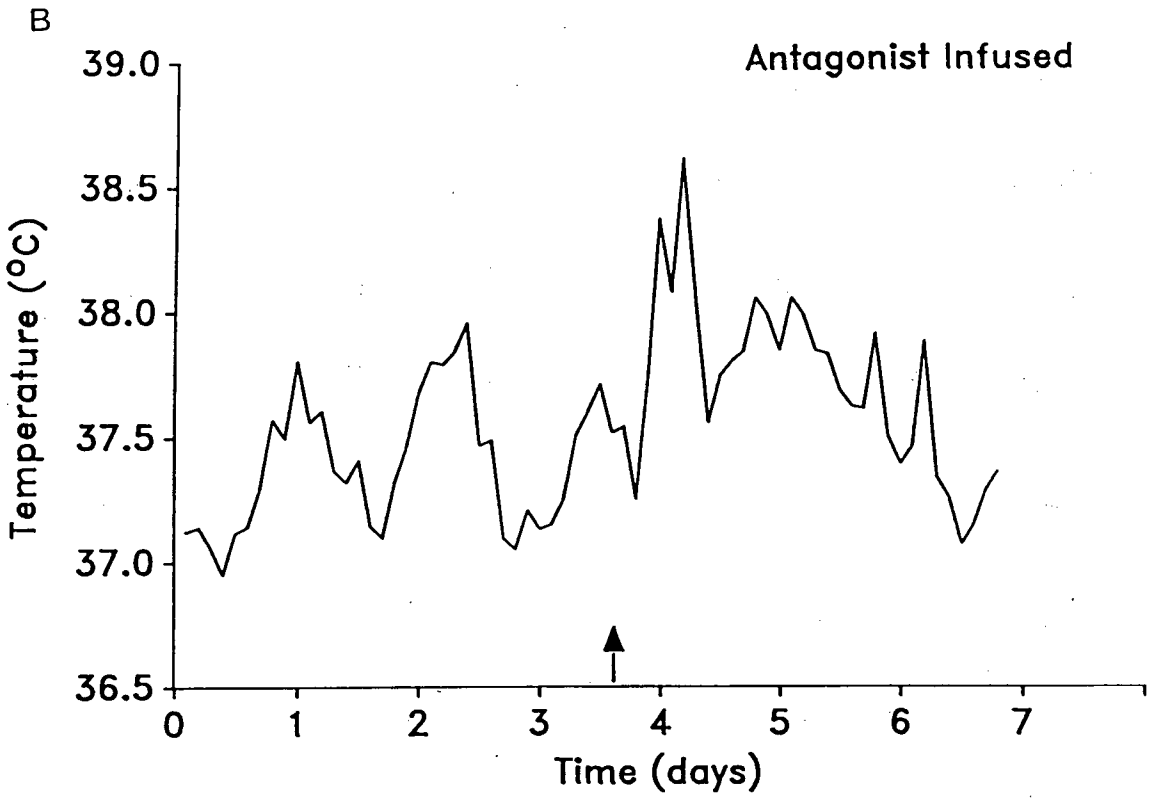
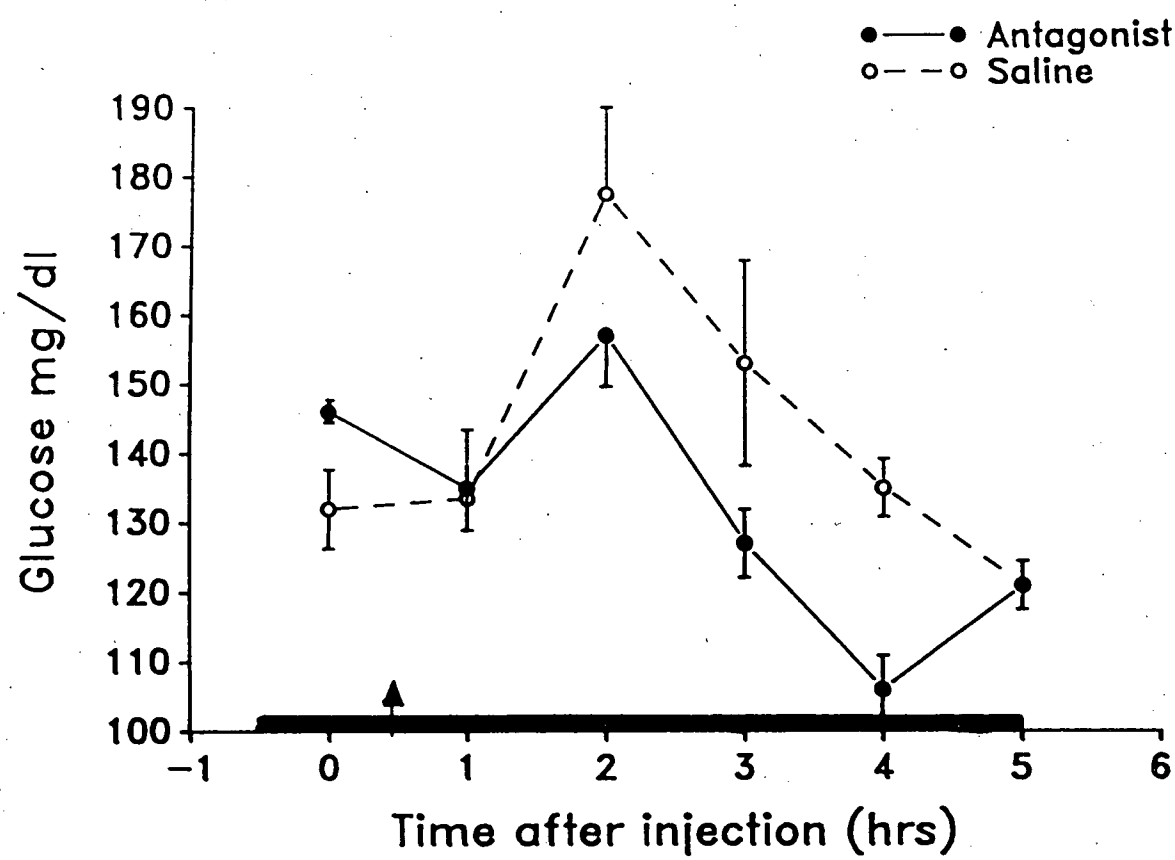

B
Antagonist Infused
39.0
38.5
38.0
37.5
37.0
36.5
Temperature (°C)
0
1
2
3
4
5
6
7
Time (days)

Figure 13

Blood glucose levels in saline infused (open circles, N = 2) or antagonist infused rats (closed circles, N = 2) after injection of bacteria (arrow). Differences between the two groups were not significantly different a $p < 0.5$. ANOVA two-way analysis of variance, $F = 3.052$, $p < 0.10$.


Antagonist
Saline
Glucose mg/dl
190
180
170
160
150
140
130
120
110
100
−1
0
1
2
3
4
5
6
Time after injection (hrs)

D. Experimental Series III

Repeated injections with antagonist infusion

Baseline body temperature between the two groups was different. The saline infused group's body temperature was approximately 0.2-0.3 ^{o}C higher than was the antagonist group before injection (Fig. 14). In the first four hour interval after injection the antagonist infused rats had a slight hypothermia as compared to the saline controls (Antagonist = 36.98 $\pm$ 0.19 ^{o}C, N = 9 vs. saline = 37.17 $\pm$ 0.17^{o}C, N = 8), though this was not significant ($p < 0.1$). Difference from baseline for the one hour interval = antagonist -0.34 ^{o}C and saline = 0.54 ^{o}C. Response during infection was not dramatically different thereafter; however, one can see from the day time six hour interval, that the antagonist infused rats responded to the same injection of bacteria with higher fevers on days 2 & 3 (Fig. 15). Day 2: antagonist = 0.85 $\pm$ 0.05, saline= 0.64 $\pm$ 0.06, Day 3: antagonist = 0.59 $\pm$ 0.14, saline = 0.36 $\pm$ 0.02, T-test; $p < 0.05$). This difference did not seem to affect survival in any significant way, as only 2/12 antagonist rats died while 1/12 saline rats died.

Control leukocyte counts were not different between the two groups (Table 1): saline = 11,662 $\pm$ 560, N = 6, antagonist = 12,315 $\pm$ 495, N = 7. One hour after injection the antagonist infused rats displayed leukopenia while the saline infused rats white blood cell count was slightly elevated (antagonist = 6,219 $\pm$ 1,430, saline = 14,883 $\pm$ 1,225, T-test;$p < 0.05$). On day 2, the antagonist infused rats had lowered leukocyte counts at both sampling times while the saline infused rats remained normal ($p < 0.05$). There was no statistical difference in leukocyte responses on day 3 between the two groups.

Figure 14

Average temperature profile over several days. Saline = open circles, N = 8, antagonist = closed circles, N = 9.

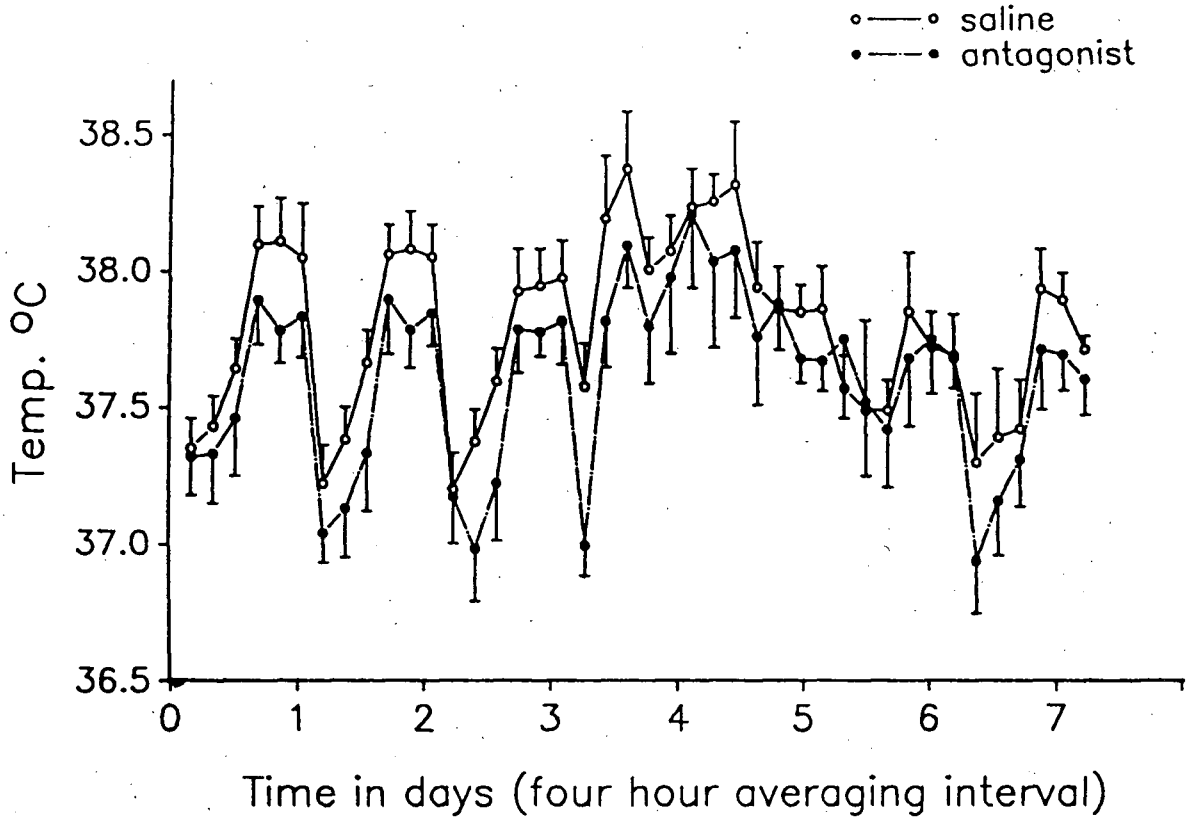

saline
antagonist
38.5
38.0
37.5
37.0
36.5
Temp. °C
0
1
2
3
4
5
6
7
Time in days (four hour averaging interval)

Figure 15

Difference from baseline for six hour daytime averaging interval. Day one is day of injection. Blank boxes = saline infused, N = 6, black boxes = antagonist infused, N = 7. Student's T-test, * $p < 0.05$.

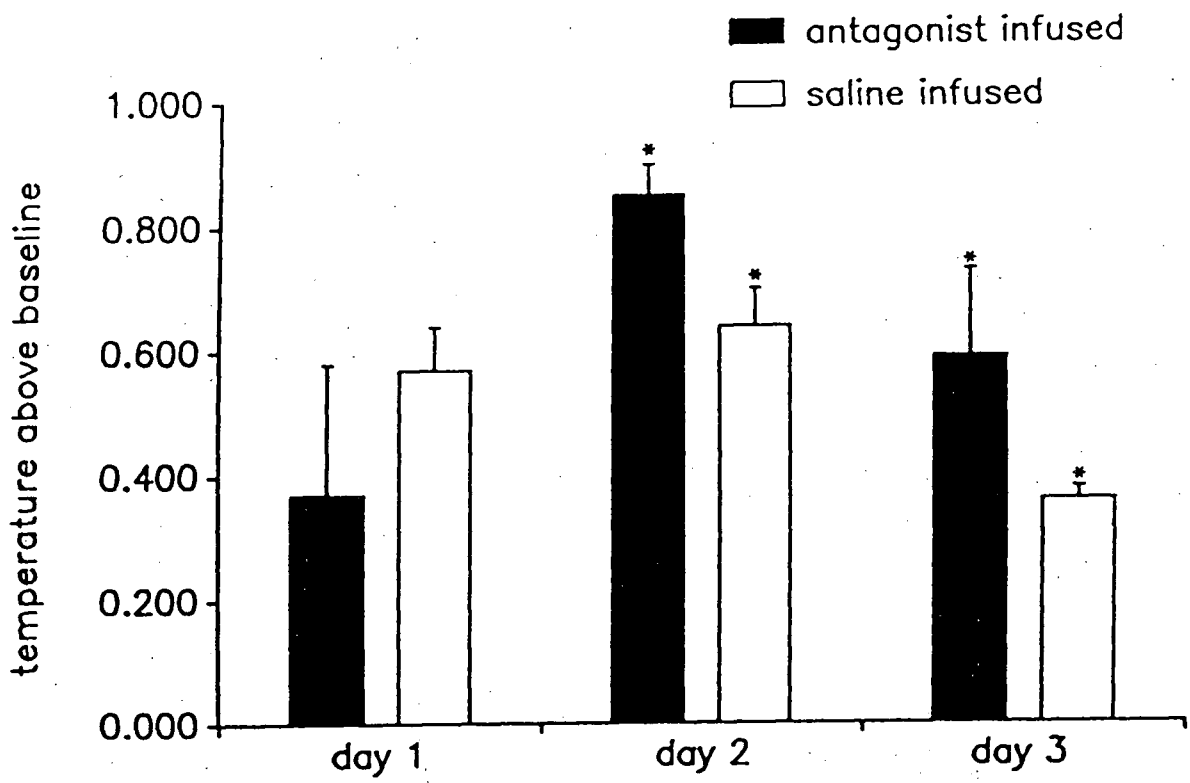

antagonist infused
saline infused
temperature above baseline
1.000
0.800
0.600
0.400
0.200
0.000
day 1
day 2
day 3

Table 1

Leukocyte responses in saline (N = 6) and antagonist (N = 7) infused rats during repeated injections of live bacteria. Infusion of V_1 antagonist or saline began one hour before injections and continued for 6 hour. Two control samples were taken for each rat. Experimental samples were taken one hour after the injection, then twice daily. ANOVA two-way analysis of variance. $F = 11.17$ (analysis between groups), $p < 0.05$.

	SALINE	ANTAGONIST
CONTROL	11,662 ± 560	12,315 ± 495
DAY 1 A.M.	14,883 ± 1225	6,219 ± 1430
P.M.	18,116 ± 4397	12,658 ± 536
DAY 2 A.M.	13,837 ± 6320	5900 ± 742
P.M.	11,633 ± 4042	8712 ± 2731
DAY 3 A.M.	11,795 ± 1842	10,116 ± 2019
P.M.	19,089 ± 2571	18,612 ± 2907

DISCUSSION

A. Infection Model

The preliminary dose response experiment did not result in a fever that continued for at least twelve hours, so we did did not deem any of the doses adequate to cause a long term infection. There was a consistent hypothermic phase that was maintained in nearly all the infection models we tested. Endotoxin recovered from gram negative bacteria administered to dogs causes a brief but significant drop in blood pressure (Brackett et al, 1983). The transient decrease in body temperature may reflect this, as the initial injection of live bacteria releases a large bolus of endotoxin. As yet, the relationship between the drop in blood pressure and drop in body temperature has not been determined, but hypothermia is a common symptom of shock. The lung model was also ineffective for our purposes.

The infection model eventually chosen was selected because it resulted in a long lasting fever. It is interesting that the fever was particularly evidenced by a loss of circadian rhythm. This suggests that the "set-point" theory of thermoregulation may be oversimplified. The normal rhythm did not occur during the period of infection as would be expected if fever were merely normal thermoregulation at an elevated temperature.

Previous experiments done with endotoxin recovered from gram negative bacteria show that AVP levels increase in the plasma significantly after injection (Kasting,1985). Plasma AVP reaches a peak at 60 minutes post-endotoxin and returns to baseline by 180 minutes. The live infection model using the same strain of bacteria shows AVP levels increasing approximately 4 hours after injection of the bacteria and

remaining elevated for up to 42 hours post injection (Fig.6, a & b). The AVP response is highly variable, that is, some rats' levels dropped back to baseline within the first few hours and some remained elevated for two days.

The AVP response to endotoxin occurs independently of the usual physiological stimuli such as hypovolemia, hypoglycemia, osmolality, increased body temperature or decreased blood pressure (Kasting et al, 1985). During this experiment, it is likely that all these parameters were being changed, so one can not rule out the possibility of any of them being contributing factors to the sustained AVP release, however; there is no reason to believe that the endotoxin may not again be the main stimulus. As the bacteria are phagocytized they release endotoxin from their inner walls, thereby providing a continual stimulus for the peripherally-released AVP. Endotoxin is not thought to cross the blood brain barrier so the central mediator for hypothalamic AVP release remains to be described. The AVP levels did not correlate in a consistent way with body temperature responses, but were back to normal by the time normal circadian rhythm was resumed.

B. Experimental Series I

Infusion of AVP

Infusion of exogenous AVP during infection results in significant alterations in the body temperature response to infection in rats. For example, there is a pronounced hypothermia seen at 12 hours after injection in the AVP infused rats. This suggests some involvement of AVP in the host defensive reaction to infection, and in fact there is much evidence to suggest that AVP is performing a beneficial role in helping to offset some of the pathological changes occuring during infection.

AVP caused a pronounced hypothermia at 12 hours. As stated earlier, release of AVP into the VSA during endotoxin fever is associated negatively with body temperature (Naylor, 1988). AVP may be released into the peripheral circulation concurrently with this central release, so that a reduction in body temperature is merely a reflection of central events. The exogenous AVP infused may exert negative feedback on the brain to prevent excessive AVP release, and after the negative inhibition is removed the brain might overcompensate with resultant hypothermia due to centrally released AVP, as was seen 12 hours after injection, since infusion stopped after 6 hours. This would also explain the slight hyperthermia seen in the first six hours in the AVP infused rats.

Another possibility is that AVP's cardiovascular effects contribute to body temperature changes. AVP is an important factor in maintenance of cardiovascular function during endotoxin or E. coli shock (Brackett et al., 1983). AVP infusions may be desensitizing the AVP V_1 receptors so that removal of the AVP results in decreased responsiveness to endogenous AVP contributing to cardiovascular maintenance. Loss of vascular tone might be reflected in a lack of ability to thermoregulate properly, resulting in hypothermia.

A third possibility is that the hypothermic effect of AVP infusion is related to its CRF activity. An increase in plasma AVP of 19.7 pg/ml is the threshold requirement for an effect on plasma corticosteroid concentration (Malayan et al, 1980). Corticosteroids have been known to be antipyretic for some time. Steroids inhibit phospholipase A which is required to convert esterified arachidonic acid to free arachidonic acid. Arachidonic acid is the precursor for prostaglandins, thought to be major contributors to the pathogenesis of fever. (Non-steroidal antipyretic drugs such as indomethacin and sodium salicylate work at a later stage in the same pathway). AVP

may thus be acting indirectly through the peripheral circulation to synergize with the central antipyretic effect.

Studies have not been conducted describing the time course of AVP action on increasing the circulating levels of corticosterone so that the hypothermia occurring at 12 hours cannot be verified to coincide with the drop in body temperature. The possibility that more than one mechanism contributes to the body temperature response to AVP augmentation during infection is very likely, particularly in light of the fact that corticosteroids substantially improve cardiovascular integrity in the presence of AVP (Brackett et al, 1983).

The glucose changes that occurred were not significantly different between groups. Glucose levels rose in both groups two hours after injection of bacteria. Endotoxin causes a transient hyperglycemia in dogs (Wolfe et al , 1977). It is likely that the glucose increases in the two groups were also endotoxin stimulated.

C. Experimental Series II

V_1 Antagonist Infusion

The V_1 antagonist blocks receptors that mediate the vasopressor, liver, and cardiac responses to AVP. Administration of the V_1-antagonist eliminates the cardiovascular maintenance effect of AVP and the gluconeogenolytic effect of AVP. If these beneficial effects are important in host defense one should see an effect on the recovery from E. coli infection. This was indeed the case, as the antagonist infused rats showed numerous alterations in the thermoregulatory response.

The antagonist infused rats showed much more variability in temperature response at six hours as indicated by the large standard of error bar (see Fig.12). This

suggests an important role of AVP in maintaining homeostasis during the acute phase reaction to bacterial challenge, as blockade of the AVP acting at the V_1 receptor reverses the response seen in experimental series I. The response at 12 hours was not different between groups. In our live infection model nighttime high body temperatures were not different from normal, thus no fever is evident 12 hours after an injection time of 11:00.

Fever has long been used as a diagnostic tool in determining the state of infection and was used as an index of recovery in this experiment. Antagonist infused rats showed a much higher fever at 24 hours then did saline controls. Apparently antagonist infusion on the day of bacterial injection prevents the ability of rats to recover as quickly as saline controls.

At 48 hours both groups were essentially back to baseline levels except one rat in the saline group which may have had a secondary infection.

AVP has stimulates bone-marrow proliferation (Perris, 1975), thymocyte proliferation (Whitfield, 1970), and gamma interferon production (Johnson et al, 1982). All of these would aid in combatting infection and be reflected in persistence of fever if antagonized.

AVP can restore or enhance RES function during conditions of shock or stress. (Altura, 1980). The RES is important in phagocytosis of bacteria. Diminished RES function results in a higher concentration of bacteria in the bloodstream. This also may be reflected in the persistent fever occurring in the antagonist-infused rats.

Three of nine antagonist infused rats died while none of the saline infused rats died. These numbers do not provide sufficient evidence to prove a necessary role of AVP in recovery from infection; however, they do suggest some involvement. Death may have occurred by a number of means as there was no time constant that would indicate blood pressure regulation or control of sepsis. One cannot rule out the

possibility that AVP's vasoconstrictor activity is of primary importance in its role in host defense. Even with non-hypotensive doses of bacteria the endotoxin released upon phagocytosis vastly increases vasopermeability therefore increases tissue damage due to loss of integrity. Again this loss of vascular tone could well be represented in the altered host response to infection as measured by body temperature.

D. Experimental Series III

Repeated Injections of Bacteria with Antagonist or Saline Infusion

If fever is used as a diagnostic tool then the fact that repeated injections of live bacteria resulted in a persistent fever would indicate a prolonged infection. This is in contrast to the tolerance usually seen in repeated endotoxin injections. This suggests that there is some stimulus other than endotoxin, that either directly or through induction of EP formation, causes a febrile response.

The antagonist infused rats had a more pronounced hypothermia in the first few hours. AVP V_1 antagonist blockade during endotoxin challenge results in an immediate fall in blood pressure (Kinter, 1986) The early hypothermia seen in the antagonist infused rats probably reflects cardiovascular changes during initial infection not compensated for by AVP acting at the V_1 receptor. A drop in blood pressure may be caused by a decrease in vascular tone so that normal sympathetic stimulation for thermogenesis, such as shivering and peripheral vasoconstriction, is ineffective. Thus, a drop in body temperature results.

AVP is a potent stimulus for gluconeogenesis and glycogenolysis in the liver acting through a V_1 receptor. Transient hyperglycemia, seen in the first few hours of infection is a characteristic response in dogs and is the result of increased glycogenolysis (Wolfe et al, 1977). Perhaps high blood glucose levels are required to provide fuel for

heat generation and removal of AVP's contribution allows body temperature to drop further than normal. The saline infused rats did have a tendency toward higher plasma glucose levels than did the antagonist group, though this was significant only to $p < 0.10$. In this experiment total glucose kinetics were not measured so a definitive role of AVP in this process cannot be ascertained.

Again , the most likely explanation for the difference seen in antagonist infused rats is that removal of AVP acting at the V_1 receptor results in changes in several physiological parameters involved in host defense, some compensated for effectively and others not. There is an interesting association between initial hypothermia and the transitory leukopenia observed in this, and other infection models. Perhaps body temperature simply reflects differences in immune response altered by the presence of AVP at the V_1 receptor.

If one looks at the difference between the two groups when a 6 hour average of daytime temperature is taken, one sees that the antagonist infused rats respond to the bacterial injections with a greater rise in body temperature. This may be attributable to a decreased immune response in the antagonist infused rats as they had slight leukopenia during the second day. As fever is a diagnostic sign of illness it appears that the antagonist infused rats do not cope with the infection as effectively as do the saline infused rats. A battery of experiments measuring plasma corticosterone, noradrenaline, adrenaline and other stress hormones would help to answer some of these questions.

Lack of any clear differences in survival between the two groups indicates that other compensatory systems are involved. There is much redundancy in the function of the "stress" hormones during infection. Angiotensin (ANG) II, adrenaline and noradrenaline all contribute to maintain cardiovascular integrity. Adrenalin, N.A., ANG II, and glucagon stimulate glycogenolysis in the liver, both independently and synergistically (Hems & Whitton, 1980). The results of this long term infection model

indicate there is enormous ability of the body to insure a specific response is available. This is illustrated by the observation that the response of the two groups is so similar.

SUMMARY STATEMENTS AND CONCLUSIONS

1) A live bacterial infection model in the rat was developed that exhibited fever as a symptom. The temperature response was significantly different than that induced by endotoxin, so that a preliminary assessment of the involvement of AVP during infection could be made.

2) AVP infusion caused a marked decrease in body temperature as compared to saline controls two hours after injection of live bacteria. Fever at 24 hours was slightly lower in the AVP infused rats. Although this indicates some physiological role of AVP, it did not significantly alter survival from E.coli infection.

3) V_1 antagonist infused prior to a single injection of bacteria resulted in an increased febrile response compared to saline controls, particularly exhibited 12 hours after injection of bacteria. Blockade of the V_1 receptor significantly alters the ability of the rats to recover from a single injection of bacteria.

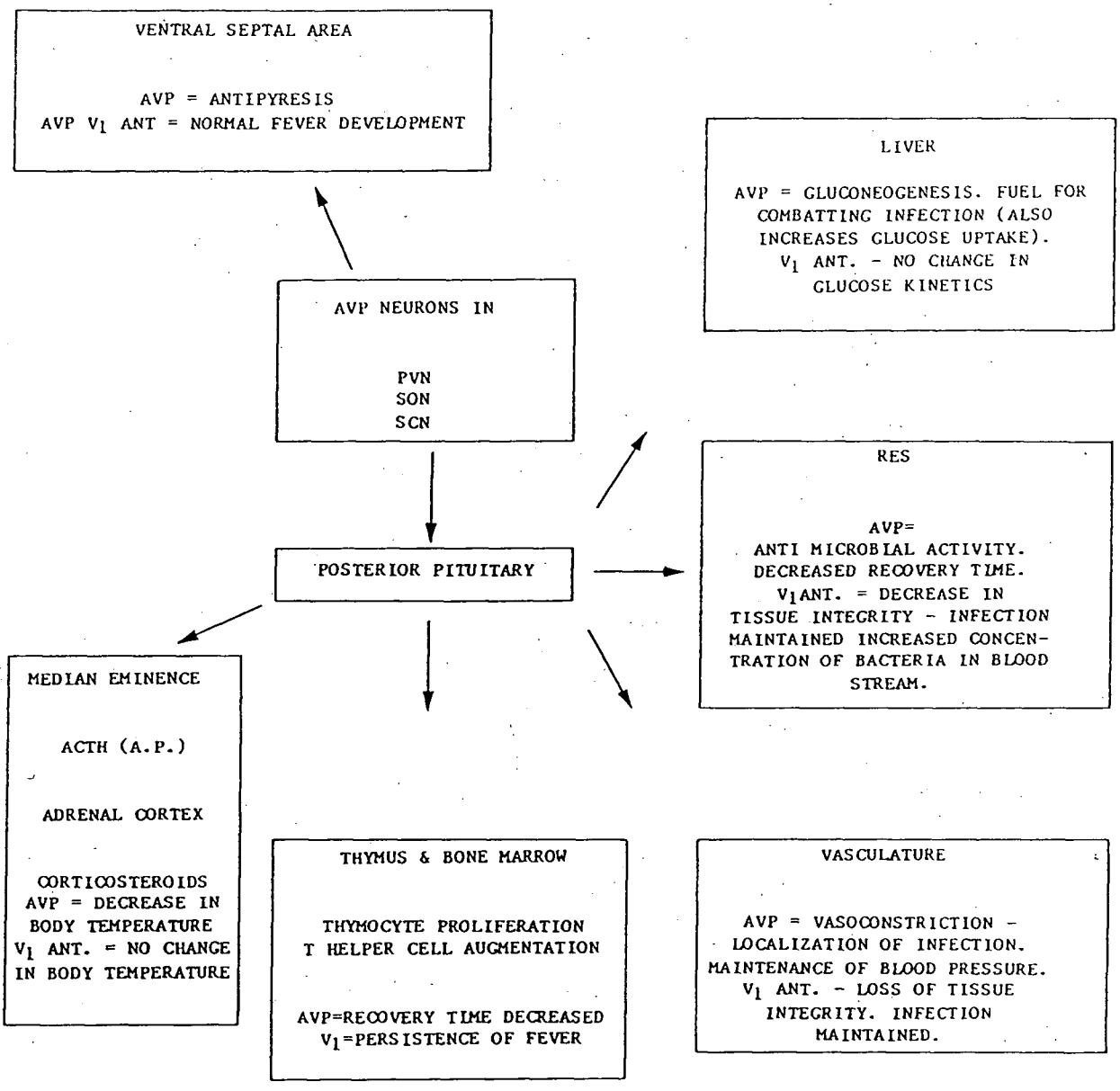
4) Repeated injections of bacteria resulted in a prolonged infection as indicated by persistent fever. This is in contrast to the tolerance seen in repeated endotoxin injections. Either the endotoxin released from the live bacteria is not as easily cleared or there are other products released by the bacteria which may elicit a febrile response.

5) V_1 antagonist infusion during repeated injections altered the course of the infection in the initial four hours and on day 2, indicating some involvement in the host defensive reaction.

6) The data presented in this thesis suggest that AVP does play a role in host defense during E. coli infection, but that other systems may provide similar functions during a longer term illness. Figure 18 shows a summary of the AVP actions that may be involved in the host defense reaction seen in this infection model.

Figure 18

Diagram summarizing the physiological effects of peripherally released AVP acting at the V_1 receptor, which may be involved in this infection model.


VENTRAL SEPTAL AREA
AVP = ANTIPYRESIS
AVP V_1 ANT = NORMAL FEVER DEVELOPMENT
LIVER
AVP = GLUCONEOGENESIS. FUEL FOR COMBATTING INFECTION (ALSO INCREASES GLUCOSE UPTAKE).
V_1 ANT. - NO CHANGE IN GLUCOSE KINETICS
AVP NEURONS IN
PVN
SON
SCN
RES
AVP=
ANTI MICROBIAL ACTIVITY.
DECREASED RECOVERY TIME.
V_1 ANT. = DECREASE IN TISSUE INTEGRITY - INFECTION MAINTAINED INCREASED CONCENTRATION OF BACTERIA IN BLOOD STREAM.
POSTERIOR PITUITARY
MEDIAN EMINENCE
ACTH (A.P.)
ADRENAL CORTEX
CORTICOSTEROIDS
AVP = DECREASE IN BODY TEMPERATURE
V_1 ANT. = NO CHANGE IN BODY TEMPERATURE
THYMUS & BONE MARROW
THYMOCYTE PROLIFERATION
T HELPER CELL AUGMENTATION
AVP=RECOVERY TIME DECREASED
V_1=PERSISTENCE OF FEVER
VASCULATURE
AVP = VASOCONSTRICTION - LOCALIZATION OF INFECTION. MAINTENANCE OF BLOOD PRESSURE.
V_1 ANT. - LOSS OF TISSUE INTEGRITY. INFECTION MAINTAINED.

REFERENCES

Altura, B.M. Evidence that endogenous vasopressin plays a protective role in circulatory shock. Role for reticulo-endothelial system using Brattleboro rats. Experientia 36:1080-1081,1980.

Atkins, E.,and Bodel, P. Clinical fever: it's history, manifestations and pathogenesis. Fed. Proc. 38:57-63,1979.

Banet, M. Fever and survival in the rat. Pflugers Arch.381:35-38,1979.

Banet, M. Fever and survival in the rat. Metabolic verses temperature response. Experientia 37:985-986,1981.

Bennett, I.L. and Beeson, P.B. The properties and biological effects of bacterial pyrogens. Medicine 29:365-400,1950.

Besedovsky, H., del Rey, A., Sorkin, E., and Dinarello, C.A. Immunoregulatory feedback between IL-1 and glucocorticoid hormones. Science 233:652-654,1986.

Brackett, D.J., Schaefer, C.F. and Wilson, M.F. The role of vasopressin in the maintenance of cardiovascular function during early endotoxin shock. Advances in Shock Research 9:147-156,1983.

Brooks, V.L. and Blakemore, L.J. Intravenous vasopressin infusion increases plasma cortisol concentration in dexamethasone treated conscious dogs. abstract In: Society for Neuroscience 18th Annual Meeting, 1988.

Buijs, R.M., Swaab, D.F., Dogterom, J. and Van Leeuwen, F.W. Intra-and extrahypothalamic vasopressin and oxytocin pathways in the rat. Cell Tiss. Res. 192:423-435,1978.

Clayton, G.W., Librik, L., Horan, A. and Sussman, L.: Effect of corticosteroid administration on vasopressin-induced adrenocorticotropin release in man. J.Clin. Endocr. 25:1156-1162,1965.

Cooper, K.E. Some physiological and clinical aspects of pyrogen. In: Pyrogens and Fever, edited by GEW Wolstenholme and J.Birch Edinburgh and London: Churchill Livingstone, pp 5-17, 1971.

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

Curnow, R.T., Rayfield, E.J., Feorge, D.T., Zenser, T.V., and DeRubertis, F.R. Altered hepatic glycogen metabolism and glucoregulatory hormones during sepsis. Am.J. Physiol. 230(5):1296-1301,1976.

Egdahl, R.H. The differential response of adrenal cortex and medulla to bacterial endotoxin. J. Clin. Invest. 38:1120-1125,1959.

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

Fernandez-Repollet, E., Opava-Stitzer, S., Tiffany, S., and Schwartz, A. Effects of endogenous antidiuretic hormone (ADH) on macrophage phagocytosis. J. Histochemistry and Cytochemistry 31:956-959,1983.

Hargrove, D.M., Bagby, G.J., Lang, C.H., and Spitzer, J.J. Adrenergic blockade prevents endotoxin-induced increases in glucose metabolism. Am. J. Physiol. 255:E629-E635,1988.

Hems, D.A., and Whitton, P.D. Control of hepatic glycogenolysis. Am Physiol. Society 60(1):17-19,1980.

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

Johnson, H.M., Farrar, W.L., and Torres, B.A. Vasopressin replacement of interleukin-2 requirement in gamma interferon production: lymphokine activity of a neuroendocrine hormone. J. Immunol. 129:983,1982.

Jones, S.B., Westfall, M.V., and Sayeed, M.M. Plasma catecholamines during E. coli bacteremia in conscious rats. Am. J. Physiol. 254:R470-R477,1988.

Kasting, M.W.and Martin, J.B. Altered release of growth hormone and thyrotropin induced by endotoxin in the rat. Am. J. Physiol. 243:E332-E337,1982.

Kasting, N.W. Simultaneous and independent release of vasopressin and oxytocin in the rat. Can. J. Physiol. Pharmacol. 66:22-26,1987.

Kasting, N.W. and Wilkinson, M.F. An antagonist to the antipyretic effects of intracerebroventricularly administered vasopressin in the rat. In: Homeostasis and Thermal Stress, edited by K. Cooper, P. Lomax, E. Schonbaum, and W.L. Veale Basel: Karger, p. 237-139,1986.

Kasting, N.W., Mazurek, M.F., and Martin, J.B. Endotoxin increases vasopressin release independently of known physiological stimuli. Am. J. Physiol. 248:E420-E424,1985.

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

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

Kinter, L.B., Dytko, G., Ashton, D.,McDonald, J., Huffman, W., and Stassen, J. Discovery and therapeutic utility of vasopressin antagonist in rats. J. of Cardiovascular Pharmacol 8(Suppl.7):S36-S43,1986.

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

Kluger, M.J., Fever and survival. Science 188:166-168,1975.

Kluger, M.J., Fever in the frog Hyla cinerea. J. Thermal Biol. 2:79-81,1977.

Kluger, M.J., Fever: it's biology, evolution, and function. Princeton University Press, Princeton, N.J., 1980.

Kovacs, G.L., and De Weid,D. Hormonally active arginine-vasopressin suppresses endotoxin-induced fever in rats: lack of effect of oxytocin and a behaviorally active vasopressin fragment. Neuroendocrinology 37:258-261,1983.

Kruk, B. and Sadowski, J. Antidiuretic action of intravenous and intracerebral pyrogen in conscious rabbits. J. Physiol. 282:429-435,1978.

Malayan, S.A., Ramsay, D.J., Keil, L.C., and Reid, I.A. Effect of increase in plasma vasopressin concentration on plasma renin activity, blood pressure, heart rate, and plasma cortico-steroid concentration in conscious dogs. Endocrinology 107(6):1899-1904,1980.

Manning, M. Lammek, B. and Kolodziejcyk, A.M. Synthetic antagonists of in vivo anti-diuretic and vasopressor response to arginine-vasopressin. J. Med. Chem. 24:701-706,1981.

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

Naylor, A.M., Pittman, Q.J., and Veale, W.L. Stimulation of vasopressin release in the ventral septum of the rat brain suppresses prostaglandin E_1 fever. J. Physiol. 399:177-189,1988.

Perris, A.D. and Hunt, N.H. Stimulation of mitosis in rat bone marrow cells by antidiuretic hormone after haemorrhage. J. Endocr. 65:2637-2646,1977.

Pittman, Q.J., Cooper, K.E.,Veale, W.L. and Van Petten, G.R. Observations on the development of the febrile response to pyrogens in the sheep. Clin. Sci. Mol. Med. 46:591-246,1978.

Reynolds, W.W., Covert, J.B., and Casterlin, M.E. Febrile responses of goldfish Carassius auratus to Aeromonas hydrophila and to Escherichia coli endotoxin. J. Fish Diseases 1:271-273,1978.

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

Stitt, J.T. Prostaglandin E. as the neural mediator of the febrile response. Yale J. of Biology and Medicine 59:137-149,1986.

Vane, J.R. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature New Biol. 231:232-234,1971.

Vaughn, L.K., Veale, W.L., and Cooper, K.E. Antipyresis: It's effect on mortality rate of bacterially infected rabbits. Brain Research Bulletin 5:69-73,1979.

Whitfield, J.F., MacManus, J.P., and Gillan, D.J. The possible mediation by cyclic-AMP of the stimulation of thymocyte proliferation by vasopressin and the inhibition of this mitogenic action by thyrocalcitonin. J. Cell Physiol. 76:65-76,1970.

Wolfe, R.R., Elahi, D., and Spitzer, J.J. Glucose and lactate kinetics after endotoxin administration in dogs. Am. J. Physiol. 232(2):E180-E185,1977.