INFLUENCE OF PRECONDITIONING TEMPERATURES AND NEUROPEPTIDES ON METABOLIC AND ENDOCRINE RESPONSES OF RATS IN THE HEAT

BY

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Increased environmental temperature produces elevated rectal temperatures (Tr) in rats. Some of the factors contributing to the elevated Tr include increased heat production, stimulation of adrenal and thyroid glands, and naturally occurring endogenous substances, such as beta-endorphin (B-END). The experiments reported herein were conducted to evaluate the effects of preconditioning temperatures and neuropeptides on the metabolic and endocrine responses of rats to varying thermal environments.

After preconditioning individually housed male Sprague-Dawley rats to temperatures of 24.5° C or 29.2° C for 14 days, animals were either exposed to experimental temperatures between 18.0° C and 34.5° C or administered B-END intracerebroventricularly (IVT) at 24.5° C in a controlled environment room. Relative humidity of 50 ± 0.3% and a 12L:12D photoperiod (L = 0900 to 2100 hr) were maintained. Metabolic rate (MR) and evaporative water loss (EWL) were measured using an

open-flow system; rectal and tail skin (Tts) temperatures using thermistors; serum corticosterone, B-END-like-immunoreactivity (B-END-LI) and thyroid hormones by radioimmunoassay. Food and water were available *ad libitum*.

Compared to 24.5° C adapted rats, rats adapted to 29.2° C maintained elevated absolute Tr and Tts, serum and pituitary gland B-END-LI, reduced serum thyroid hormones and comparable serum corticosterone levels. The thermoneutral zone of these two groups of rats was different. At temperatures above 29.2° C, changes in Tts and EWL were of a greater magnitude for the 24.5° C adapted rats compared to the 29.2° C group. The ΔTr of rats preconditioned to 24.5° C and exposed to 32.5° C were similar to those produced by IVT injections of B-END at 24.5° C; the concentration of B-END-LI in the bloodstream of these two groups of rats was similar. Beta-endorphin injected IVT produced a dose-related hyperthermic response that was antagonized by naloxone but not adrenergic antagonists.

These data indicate that the response of rats to changing environmental conditions is influenced by their thermal history. Preconditioning temperatures may alter the reference point around which Tr is regulated. Beta-endorphin appeared to elevate Tr centrally, though μ opiate receptors. Additionally, stimuli which released B-END-LI into the bloodstream may also increase the synthesis or delivery of B-END-LI to the pituitary gland. Beta-endorphin appears to be involved in physiological adaptation of rats to heat.

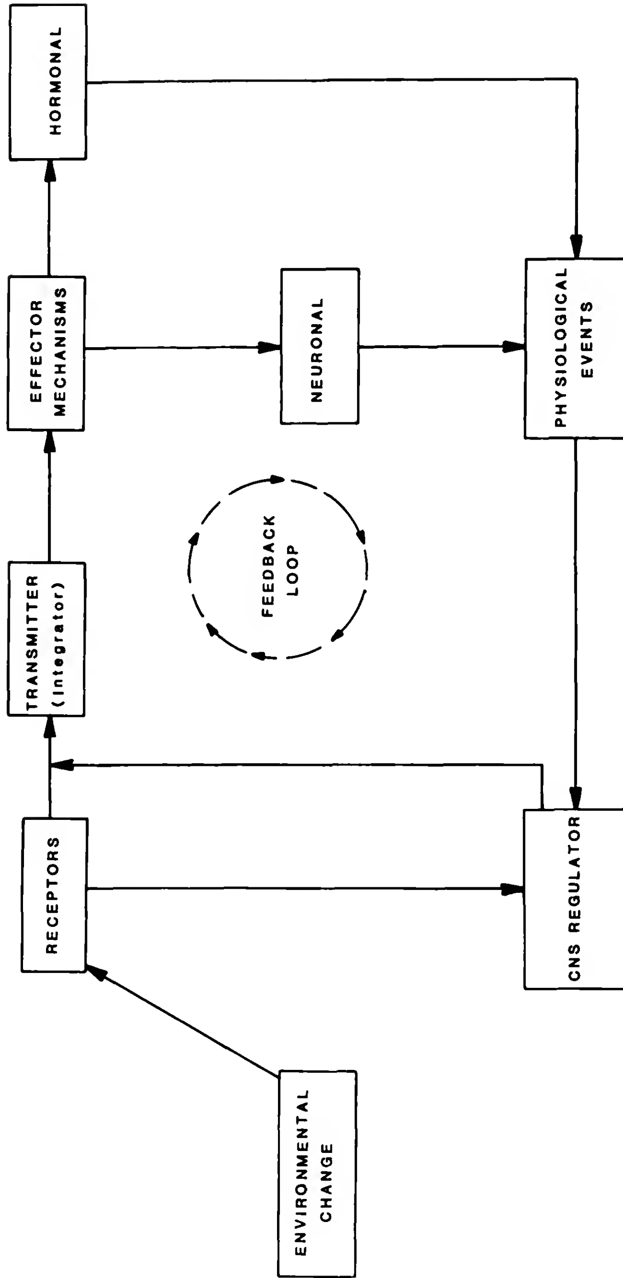
INTRODUCTION

When an animal is exposed to an extreme change in environment, a process of adjustment or physiological adaptation results (Prosser, 1964). Although the term "adaptation" includes genetic heritage and natural selection, "physiological adaptation" refers to the readjustment of the animal to itself, other organisms and/or to its surrounding environment. Another term, acclimatization, is used to describe the long term physiological adjustments induced by complex environmental factors such as seasonal and climatic changes; acclimation or conditioning refers to changes in response to a single variable, as in a controlled experiment (Hart, 1957).

Adolph (1964) proposed a generalized scheme to describe the adaptation process (Figure 1). A change in the animal's thermal environment is sensed by temperature receptors (located on the skin, etc.) which transmit this information to a transmitter which relays this message to an effector organ (vasomotor, metabolic, sudomotor). Through hormonal or neural mechanisms, the effector organ responds with an appropriate output (vasodilation, sweating, etc.). This response is coordinated by the central nervous system regulator (hypothalamus) that maintains body temperature at a predetermined ("set-point") level and is influenced by feedforward and feedbackward information. As a result, the organism is able to respond appropriately to the "new" environment. Physiological adaptation develops slowly on exposure to

Figure 1. Schematic of the events involved in the process of physiological adaptation. (See text for details.)

SOURCE: Adolph (1964).


ENVIRONMENTAL
CHANGE
RECEPTORS
CNS REGULATOR
TRANSMITTER
(Integrator)
FEEDBACK
LOOP
EFFECTOR
MECHANISMS
NEURONAL
PHYSIOLOGICAL
EVENTS
HORMONAL

the new environment and disappears slowly when the stress is removed. These processes are called adaptation and deadaptation, respectively.

The center of the thermoregulatory system is believed to be the preoptic area of the anterior hypothalamus (POAH). Thermosensitive neurons in this area receive afferent input from cutaneous thermoreceptors (Pierau and Wurster, 1981) and from deep body thermosensitive neurons in the spinal cord (Boulant and Hardy, 1974; Guieu and Hardy, 1970), posterior hypothalamus (Nutik, 1973; Wunnerberg and Hardy, 1972) or other sites within the central nervous system (Mercer and Jessen, 1978). Afferent information from peripheral thermoreceptors is relayed through unmyelineated C fibers and thinly myelinated A delta fibers which are sensitive to heat and cold, respectively. This skin temperature information travels upward with pain information through the lateral spinothalamic tract (Brodal et al., 1967). This pathway projects to the ventrobasal thalamus with collateral projections to neurons in the reticular formation (Nakayama and Hardy, 1969), midbrain and raphe nuclei (Dickenson, 1976) which relay this thermosensory information to areas that include the POAH (Fuxe, 1965). Other extra hypothalamic areas also may be involved in temperature regulation (Boulant, 1981).

The thermosensitive neurons in the POAH integrate hypothalamic thermal information with thermal information from peripheral and deep body structures. This includes information from thermally active endogenous substances as well as local neuronal input. The sensitivity of the body to heat may be reflected by the 3:1 ratio of warm to cold sensitive neurons (Boulant, 1981). As a result of this integration, appropriate thermoregulatory responses are evoked through efferent

pathways to lower regions of the brain stem and spinal cord (Saper et al., 1976) for maintenance of body temperature.

Body heat loss and heat production responses depend on the degree of neuronal stimulation or temperature change of the POAH. The magnitude of this response is proportional to the change sensed by the POAH (Boulant, 1981). Feldberg and Myers (1963) reported the balance of norepinephrine and serotonin in the POAH may determine "set-point" temperature. This is supported by Hellon (1981) because the raphe nuclei is the only source of serotonin containing neurons in the brain. Fibers in this area may relay thermal information to the POAH using serotonin as a neurotransmitter. Direct heating of the POAH may inhibit the release of thyroid releasing and stimulating hormones and, consequently, the release of thyroid hormones (Andersson et al., 1962). Lomax and Green (1981) found biochemical evidence that histamine may be a neurotransmitter in the POAH. Opioid peptides may act on the thermoregulatory system through serotonin (Lin et al., 1979; Martin and Bacino, 1979), dopamine (Lal et al., 1976; Martin et al., 1980), acetylcholine (Yehuda and Kastin, 1980) or prostaglandin E_2 (Martin et al., 1977). These and other hormones or endogenous substances may alter body temperature by acting on any part of the thermoregulatory system together or alone.

Thermoregulation enables homeotherms to maintain constant body temperatures within a range of environmental temperatures. The thermal state of the environment stimulates physiological, behavioral and morphological responses and determines heat transfer between the environment and the animal. Important elements in this transfer are environmental dry bulb temperature, air humidity, radiant temperatures

(infrared and solar radiation) and air movement (Ingram and Mount, 1975).

Between the extremes of hot and cold environments lies a range of temperature in which homeotherms maintain a relatively constant body temperature; this is the zone of homeothermy. Within this zone is a smaller range of environmental temperatures in which an animal maintains a relatively constant body temperature with minimal adjustments in metabolic rate; this is the zone of thermal neutrality. The latter zone is bounded by the upper and lower critical temperatures--for heat and cold, respectively (Hart, 1957), and varies with the age, sex and species of the animal (Mitchell and Carman, 1926; Benedict and MacLeod, 1929; Gelineo, 1964; Folk, 1974). Above and below the critical temperatures an animal increases metabolic rate to prevent a change in body temperature.

Maintenance of a relatively constant body temperature is a balance between heat loss and heat producing mechanisms (Table 1). The balance between these two factors may be altered by changes within the body or by the external environment. The resultant response is controlled by the central thermoregulatory system which involves endogenous substances at each step in the process described above. Substances that have been shown to alter the thermoregulatory processes include acetylcholine, serotonin, dopamine, histamine, norepinephrine, adrenocorticotropic hormone (ACTH), beta-endorphin (B-END), bombesin, arginine vasopressin, neurotensin, glutamate, aspartate and taurine (Blatteis, 1981).

Heat may be lost to the environment through conduction, convection, radiation or evaporation. However, the amount of heat lost by

Table 1. Factors contributing to heat balance in homeothermic animals.

FACTORS INCREASING HEAT PRODUCTION (over basal metabolic rate)	FACTORS DECREASING HEAT PRODUCTION
1. Exercise or shivering	1. Chemical decrease of metabolic rate (thyroid hormones, catecholamines)
2. Imperceptible tensing of muscles	2. Decreased activity
3. Chemical increase of metabolic rate (thyroid hormones, catecholamines)	3. Decreased food intake
4. Specific dynamic action of food (hunger)	
5. Disease (fever)	
6. Nonshivering thermogenesis	
FACTORS DECREASING HEAT LOSS	**FACTORS ENHANCING HEAT LOSS**
1. Shift in blood distribution (vasoconstriction)	1. Sweating
2. Decrease in tissue conductance	2. Panting
3. Counter-current heat exchange	3. Cooler environment
4. Piloerection	4. Increased skin circulation (vasodilation)
5. Behavioral adjustment (curling up)	5. Decreased clothing or shorter fur insulation
	6. Increased insensible water loss
	7. Increased radiating surface
	8. Increased air movement (convection)
	9. Behavioral adjustment (laying "spread-eagle")

SOURCES: Folk (1974); Ganong (1977).

each of these means varies with ambient environment conditions. Heat is lost by conduction through physical contact of the animal with air and objects at a lower temperature. Loss of heat by conduction is minimized by insulation with fur and clothing. Convection is similar to conduction and is sometimes referred to as air conduction. Radiation is the loss (or gain) of heat in the form of electromagnetic infrared heat waves. These avenues all contribute to sensible or non-evaporative heat loss (Folk, 1974).

On the other hand, evaporative heat loss--also called latent loss--involves a change of phase of water from the liquid to the gaseous state. Latent loss usually involves perspiration (sweating) or increased evaporation from the lungs (panting). In the latter, shallow breathing increases the amount of water vaporized in the mouth and respiratory passages and is an effective heat loss mechanism. So called "insensible heat loss" is a form of latent heat loss and results from continual diffusion of water molecules through the skin and respiratory tract. Other losses of heat through the skin depend on the skin temperature, which is regulated by tissue conductance, blood flow (vasoconstriction and vasodilation) and evaporation (Yoshimura, 1964). Evaporative heat loss is an effective thermoregulatory mechanism because 0.58 cal of heat is lost for each gram of water evaporated from the body surface (Gelineo, 1964; Folk, 1974).

Under elevated temperatures, an animal may gain heat from the environment but generally loses heat via physiological and behavioral mechanisms (e.g., panting, burrowing and vasodilation). The ability of animals to survive in the heat depends upon their ability to both dissipate heat and maintain water balances within their bodies.

Some animals such as the kangaroo rat have the ability to utilize metabolic water and excrete a more concentrated urine (Schmidt-Nielsen, 1979).

Morphological changes during prolonged exposure to heat include thinning of the fur (Gelineo, 1949) and increase in the relative length of the tail (Sumner, 1909; Sundstroem, 1922; Heroux, 1961). Behavioral adaptations include laying "spread-eagle" to increase surface area in addition to burrowing and estivating, as seen in some desert species (Schmidt-Nielsen, 1964).

Many mammals, such as the rat, do not sweat or pant in the heat but increase heat loss by regulating the flow of blood to the tail (Rand et al., 1965), through evaporation of water from their respiratory tract, and by spreading saliva on their body surfaces (Hainsworth, 1968). The tail of the rat may account for as much as 10% of the cardiac output or for dissipating from 5-25% of the rat's metabolic heat production (Rand et al., 1965). Heat also may be lost through the ears and feet (Dawson and Keber, 1975), although Grant (1963) and Gemmell and Hales (1977) demonstrated that the ears of the rat do not function in response to thermal stimuli as do the ears of other animals such as the rabbit. Moreover, heat loss from nude areas of the rat other than the tail is insufficient alone to support thermoregulation and is accompanied by increased evaporative water loss (EWL) in the heat (Stricker and Hainsworth, 1970). Evaporation accounts for more than 49.2% of the heat lost by rats at 33° C (Swift and Forbes, 1939; Hainsworth and Stricker, 1970). Male rats also groom saliva on the testes and scrotum which increases the surface area for evaporation (Herrington, 1940; Hainsworth, 1967), survival time in the

heat (Stricker et al., 1968), and may account for the ability of males to dissipate heat more effectively than females (Hainsworth, 1968). The mechanisms of heat loss through the tail and by evaporation are interdependent: removal of either impairs the rat's ability to regulate body temperature, while removal of both leaves the rat vulnerable to heat stress (Stricker and Hainsworth, 1971).

Yousef and Johnson (1967) reported initial exposure of rats to heat is marked by an increased rectal temperature (Tr) and metabolic rate (MR). Elevated MR is commonly seen when animals are exposed to conditions for which they are unaccustomed or nonadapted (Herrington, 1940; Yousef and Johnson, 1967) and signals the start of physiological adjustment to the new environment (Prosser, 1964). When Tr reaches 38.5° C saliva secretion begins (Stricker and Hainsworth, 1970). Circulating catecholamines from the adrenal medulla also may stimulate salivary secretion as intravenous (IV) administration of epinephrine elicited submaxillary saliva secretion in rats (Elmer and Ohlin, 1971). The saliva is groomed onto body surfaces and through evaporation helps prevent further increases in Tr (Hainsworth, 1968). Malan and Hildwein (1969) observed increased EWL at a Tr of 39.5° C. At a Tr of 39.2° C (Little and Stoner, 1968) and hypothalamic temperature (Thy) between 37.5° C and 39.0° C (Young and Dawson, 1982) vasodilation of the rat tail occurs. This response continues until Thy falls by 0.2-0.4° C, at which time vasoconstriction occurs until Thy rises again (Young and Dawson, 1982).

After several hours (6-18 hr) in the heat, Tr reaches maximal level plateaus and is maintained at a new elevated level 0.5-2.0° C above control values (Yousef and Johnson, 1967; Horowitz, 1976).

Tail skin temperature (Tts) increases to 0.5-3.0° C below Tr (Johanssen, 1962). The temperatures of the fore and hind limbs act in a similar manner (Gemmell and Hales, 1977). Metabolic rate remains elevated from 48 hr (Yousef and Johnson, 1967) to several weeks (Gelineo, 1934; Beattie and Chambers, 1953) after which time MR declines to control values.

The elevated MR observed on initial exposure to heat may be attributed to increased secretions from the adrenal and thyroid glands or increased muscular exertion of respiration (Yousef and Johnson, 1967). Glucocorticoids have been shown to exert a significant calorigenic effect in rats (Evans et al., 1957). Kotby and Johnson (1967) found elevated plasma corticosterone levels in rats after 1 hr in the heat and maximum levels of this hormone were reached after 24 hr at 34° C. Similar findings were observed in this laboratory (unpublished observations).

All of these hormones are among those that regulate energy metabolism (Collins and Weiner, 1968). Jones et al. (1976) reported decreased utilization of norepinephrine by heat adapted animals, suggesting decreased sympathetic activity accompanies heat adaptation. Adrenocortical hormones regulate glucose-6-phosphatase activity (Collins and Weiner, 1968). Chayoth and Cassuto (1971a, b) reported reduced activity of this enzyme in heat acclimated hamsters. Other biochemical adaptations include reduced rates of mitochondrial respiration and alterations in carbohydrate and fat metabolism in heat producing organs (liver, kidney and brown adipose tissue) of heat acclimated hamsters (Cassuto, 1968, 1970; Cassuto et al., 1970; Chayoth and Cassuto, 1971a, b; Inbar et al., 1975; Rabi and Cassuto, 1976).

Such biochemical adaptations are directed toward lowering the activity of energy producing metabolic pathways (Cassuto, 1968) and may be reversed by administration of triiodothyronine to heat-acclimated animals for at least 3 days (Cassuto et al., 1970).

Adrenal and thyroid hormones may act at regulatory site(s) of these metabolic pathways to aid in chemical thermosuppression (Cassuto, 1968) and decrease metabolic heat production in rats in the heat. Physiological adaptation to heat is accompanied by reduced thyroid function (Dempsey and Astwood, 1943) and adrenal cortical activity (Kotby and Johnson, 1967). Yousef and Johnson (1968) found shifts in the amounts of iodine compounds in the plasma of heat acclimated rats: the relative amount of thyroxine decreased, while that of free iodide and monoiodotransferase increased during heat acclimation. These hormonal and biochemical responses occur jointly with the lowered MR observed after exposure to heat for 10 days.

Other physiological adjustments on exposure to heat include a rise in total daily water consumption (Hainsworth, 1968; Attah and Besch, 1977; Gwosdow-Cohen, 1980) which may replace water lost by evaporative cooling (Hainsworth, 1968; Attah and Besch, 1977; Gwosdow-Cohen, 1980). Total daily food consumption is decreased (Brobeck, 1960; Hamilton, 1963; Attah and Besch, 1977; Gwosdow-Cohen, 1980) and may be a factor in reducing thyroid activity in the heat (Yousef and Johnson, 1968; Valtorta et al., 1982). Fluid shifts between compartments (Jones et al., 1976; Horowitz, 1976) and an increased body water turnover (Attah and Besch, 1977; Gwosdow-Cohen, 1980) are found in these animals. Jansky and Hart (1968), Jones et al. (1976) and Bobeck et al.

(1980) reported a redistribution of blood flow away from heat producing organs, including the thyroid gland.

Exposure to heat also causes reduced growth rates of rats (Ray et al., 1968). Body weight initially decreases during the first 3 days of heat exposure but increases after this time (Horowitz and Soskoline, 1978). The heat decreases the weights of most organs (Ray et al., 1968; Jones et al., 1976) with the exception of the salivary glands which begin to enlarge within 48-96 hr after heat exposure (Elmer and Ohlin, 1969, 1970; Horowitz, 1976). Salivary gland enlargement serves as a mechanism to increase saliva production for EWL in the heat (Horowitz and Soskoline, 1978). These manifestations of heat adaptation are completed in 7 to 10 days (Yousef and Johnson, 1967; Horowitz, 1976) and within 2 to 4 weeks return to original levels (Gelineo, 1934; Sellers et al., 1951; Folk, 1974).

Gelineo (1934) and Schwabe et al. (1938) reported that MR (measured as oxygen consumption) of rats was influenced by the temperature at which animals were physiologically adapted. Rats adapted to heat (30-32° C) consumed less oxygen at 5° C than rats adapted to cold (0-2° C). These cold adapted rats regulated Tr at -10° C while heat adapted animals became hypothermic at temperatures below 25° C. Cold acclimated rats also survived longer at -10° C compared to warm acclimated animals (Hart, 1957). In contrast, Cottle and Carlson (1954) found no effect of thermal history on MR of 5° C and 25° C adapted rats.

Rand et al. (1965) showed that thermal history influenced the critical temperature for vasodilation of the rat tail. Lewis et al. (1960) were unable to dehydrate the same rat at different ambient

temperatures due to physiological adaptation. Gwosdow-Cohen (1980) reported that preconditioning temperature (24.5° C or 29.2° C) and route of exposure (direct or stepwise) influenced the rat's EWL, body water turnover, food intake and water intake responses to prolonged (2 weeks) heat exposure. Previous studies of thermal history emphasized individual thermoregulatory responses, and although these responses are important, interactions of these and other adjustments ultimately determine the rat's response to thermal stress. However, no studies have been conducted to measure the influence of thermal history on MR, EWL, Tr and Tts in the same rat.

Changes in the endocrine state of rats preconditioned to different environmental temperatures may be responsible for thermoregulatory alterations which could provide information on possible mechanisms of action. Since ACTH and B-END are derived from the common precursor, preopiomelanocortin (Blatteis, 1981), and released from the pituitary gland together (Guilleman et al., 1977; Csontos et al., 1979; Mueller, 1980) on exposure to heat (Deeter and Mueller, 1981), and in response to stressful stimuli (Millan et al., 1981; Mueller, 1981), it seems likely that both of these hormones may be involved in the physiological response to heat. The amount of B-END released is directly proportional to ACTH levels in blood (Kreiger et al., 1979). In addition, physiological levels of B-END produce hyperthermia (Blasig et al., 1979; Thornhill and Wilfong, 1982) and appear to increase the "set-point" around which body temperature is regulated, regardless of ambient temperature (Bloom and Tseng, 1981; Clark, 1981).

Lin et al. (1979) suggest that at 22° C or below, B-END increases sensible heat loss and decreases MR. Alternatively, naloxone was

reported to increase body temperature in rats exposed to the heat (Holaday et al., 1978b; Thornhill et al., 1980) which infers that B-END may prevent changes in body temperature on exposure to heat. The ability of B-END to alter body temperature suggests involvement in the process of physiological adaptation. Mobilization of B-END stores may be influenced by environmental temperature which directly or indirectly produce body temperature changes seen on exposure to different environmental temperatures. These considerations led to the experiments reported here.

CHAPTER 1
THERMOREGULATORY CHANGES IN RATS PRECONDITIONED TO DIFFERENT ENVIRONMENTAL TEMPERATURES

Introduction

The influence of thermal history on the physiological adaptation of rats in the cold has received more attention than rats in the heat (Hart, 1957). Rand et al. (1965) and Thompson and Stevenson (1966) showed that acclimatization temperature influenced the temperature for vasodilation of the rat tail. In addition, Lewis et al. (1960) were unable to dehydrate the same rat at different ambient temperatures due to physiological adaptation.

For rats adapted to environmental temperatures between 0-25° C, the upper critical temperature remains relatively constant at 29-30° C (Swift and Forbes, 1939; Herrington, 1940) but increases to 34.5° C for rats adapted to 30-32° C (Gelineo, 1934). Poole and Stephenson (1977) suggested 18-28° C as a thermoneutral zone and this commonly is used as the range of animal room temperatures for the housing of rats. On the other hand, reproductive and growth differences in rats housed within this temperature range have been reported (Yamauchi et al., 1981), and 20-26° C was suggested as the optimal temperature range for the housing of rats. Benedict and MacLeod (1929) reported metabolic rate (MR) differences in rats housed at environmental temperatures as little as 3° C apart.

Significant increases in water intake and body water turnover have been reported (Attah and Besch, 1977) in rats physiologically adapted to 29.2° C and 34.0° C compared to 24.5° C. Yet, for rats preconditioned to room temperature (25.0° C), evaporative water loss (EWL) does not increase until the ambient temperature exceeds 33.0° C to 34.0° C (Hainsworth, 1968). Other evidence (Hainsworth and Stricker, 1970) suggests that increased salivary secretions in rats at 32° C are the primary contributors to EWL between 29-36° C.

What is not clear from prior research is the role of preconditioning environmental temperature on the relationship between the rat's MR, shifts in upper critical temperature, and EWL. Previous reports emphasized responses to changes in single variables and, although these responses are important, interactions of multiple adjustments ultimately determine the response of the animal to thermal stress. Hence, the present study was conducted to determine the influence of preconditioning temperatures on the rat's responses (MR, EWL, and rectal and tail skin temperature) to elevated temperatures. The results are reported here.

Materials and Methods

Animals

Sixty adult (3 months old), male Sprague-Dawley rats [Caw: CFE (SD)] were used in these experiments. These rats (416.3 ± 8.6 g, mean ± S.E.) were housed individually in metabolic cages (Model 4-641-000, Acme) and were fed pulverized rat food (Laboratory Rodent Chow, Ralston Purina) from feed cups with perforated inserts to

minimize spillage. Water bottles were equipped with sipper tubes and attached to each cage. Food and water were available *ad libitum*. Use of duplicate feed cups and water bottles allowed for rapid daily interchange, caused minimal disturbance to the rats, and provided an efficient procedure for quantifying food and water intake (Besch, 1970).

Environmental Room

Two environmental chambers (Model C7-88, Forma Scientific) were used in this study: one for preconditioning and one for thermal exposures. Dry-bulb temperatures of 24.5 ± 0.1° C or 29.2 ± 0.1° C were utilized as preconditioning temperatures. Relative humidity of 50 ± 0.3% and a 12L:12D photoperiod (L = 0900 to 2100 hr) were maintained. Continuous monitoring of the ambient temperature in the environmental room was accomplished through thermistors (Model 43TD, Yellow Springs Instruments) interfaced with a data acquisition system (Model PD2064, Esterline Angus).

Experimental Design and Procedure

Rats were exposed to either 24.5 or 29.2° C for at least 2 weeks before beginning each experiment. Two weeks were allowed for physiological adaptation because of previous reports (Gelineo, 1934; Cassuto and Chaffee, 1963; Yousef and Johnson, 1967; Folk, 1974; Horowitz, 1976; Horowitz and Soskoline, 1978) that primary changes associated with long term physiological adaptation are completed in 10 days. Furthermore, when the experiments described herein were replicated with

rats preconditioned for 6 weeks, quantitatively similar results to those reported were obtained.

After this adjustment period, the rats were randomly selected and paired for acute (3 hr) exposure to one of the experimental temperatures ranging between 18.0 and 34.5° C. The order of exposure of each rat to each temperature remained constant throughout the experiment and was separated by 3-5 days. Measurements of rectal (Tr) and tail skin (Tts) temperatures and body mass were made at the beginning and end of each 3 hr experimental session. Evaporative water loss and MR for each rat were measured at each temperature.

Measurements

Measurements were performed between 0900 and 1200 hr.

Food and water intake. Food and water intake and body mass were measured using a top loading balance (Model P1200N, Mettler). Food and water intake measurements were calculated as the difference in weight of the respective containers between the beginning and end of each 24 hr period.

Body temperature. Rectal temperature was measured using a thermistor (Model 402, Yellow Springs Instruments) inserted 5 cm into the rectum. Tail skin temperature was measured with a thermistor (Model 409, Yellow Springs Instruments) positioned 2.5 cm from the base on the ventral surface of the tail (Young and Dawson, 1982). Both thermistors were held in place with gauze tape wrapped around the tail. Each thermistor was connected to a telethermometer (Model 44TD, Yellow Springs Instruments) for visual display of the temperature. Rectal and

tail skin temperature differentials were computed as the difference in Tr and Tts, respectively, at the beginning and end of each experimental session.

Evaporative water loss. The open-flow system of Hainsworth (1968), as modified by Gwosdow-Cohen (1980), was used for determination of EWL in rats. Simultaneous measurements were made on two rats one in each of two cylindrical (height 26 cm; diameter 22 cm), 8-liter polypropylene containers (Model 5304, Nalgene) with slip-on lids (Model 5308, Nalgene) as illustrated in Figure 2. To complete the assembly, the containers were placed in a plywood frame that was secured with an adjustable strap and attached to the system detailed in Figure 3.

To assure an air-tight container, a 2 cm wide strip of foam insulation tape was glued to the lid to provide a seal between the lid and the walls of the container. A wire mesh grid was attached to each lid to prevent the rats from chewing the tape. The lid of each container was equipped with two sealed tube connector fittings (Model 4-HC-A-401, Cajon) which served as air inlet and outlet openings. A 12.5 cm galvanized steel pipe extension was attached to each connector fitting to deliver air into the middle of each container through plastic (Tygon) tubing. A third opening in the lid was equipped with a quick-disconnect fitting (Model QC4-B-4PM, Swagelok) which allowed for monitoring of container temperature and pressure. No temperature or pressure changes between container and outside were detected in any of the experiments. Each container was equipped with a wire mesh grid placed 5 cm above the container floor. A 2.5 cm layer

Figure 2. A detailed diagram of the containers used for evaporative water loss and metabolic rate measurements.

(See text for details.)

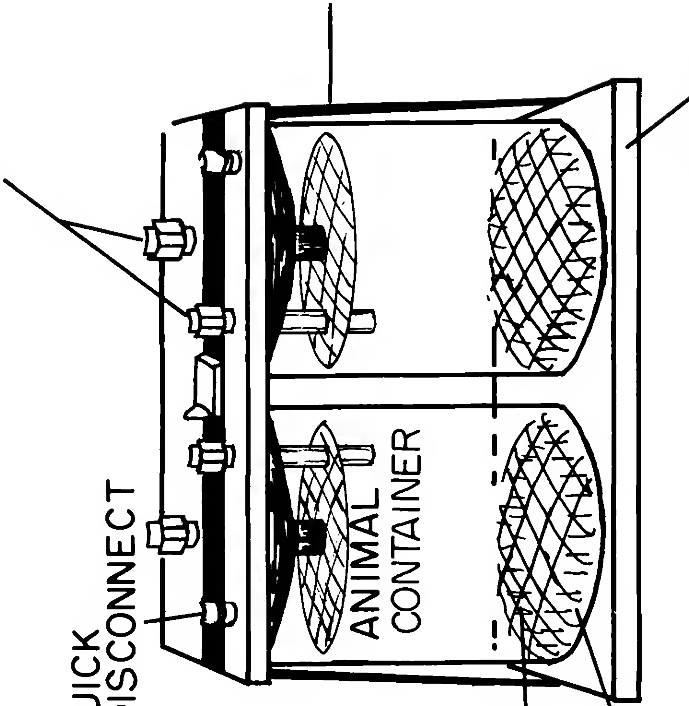
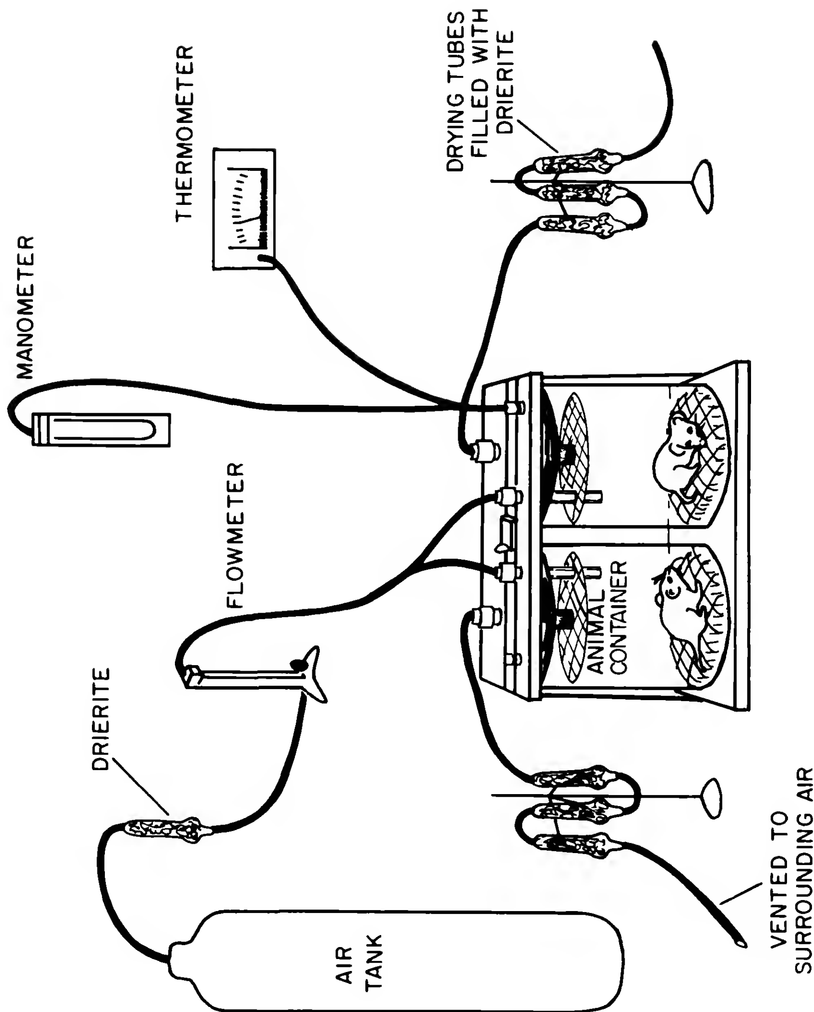

AIR INLET AND OUTLET
ADJUSTABLE STRAP
WOOD FRAME
QUICK DISCONNECT
ANIMAL CONTAINER
WIRE MESH
MINERAL OIL

Figure 3. Schematic of the apparatus used to measure evaporative water loss.

For sake of simplicity, the manometer and thermometer are shown attached to only one container although pressure and temperature measurements were made in each container. (See text for details.)


MANOMETER
THERMOMETER
DRYING TUBES
FILLED WITH
DRIERITE
FLOWMETER
DRIERITE
ANIMAL
CONTAINER
AIR
TANK
VENTED TO
SURROUNDING AIR

of mineral oil (Mallinkrodt) on the floor of each container prevented urinary and fecal moisture from contributing to EWL measurements.

Before EWL data were collected, a period of 25 min was allowed for the development of the saliva spreading and Tr responses in each rat (Hainsworth, 1968). Tanked dry air was passed through a tube containing loosely packed anhydrous calcium sulfate (Drierite, Hammond Drierite Co.) and a flowmeter (Model 100H, Lab-Crest Century) at a constant rate of 8 liter/min before entering the sealed containers (i.e., 4 liter/min through each container). Air exited from each container through three drying tubes (12.7 cm each) containing loosely packed anhydrous calcium sulfate to absorb the water vapor for a 15 min collection period. The tubes then were removed from the environmental chamber and allowed to equilibrate with room temperature (24.5° C) for 10 min before weighing to the nearest 0.01 mg on an analytical balance (Model 2474, Sartorius). The drying tubes were handled with gloves at all times. Evaporative water loss was determined by the change in weight of the tube between the beginning and end of the 15 min collecting period and calculated as water loss per unit of body mass per hour ($mg/g \cdot hr^{-1}$). Data from this collection period represent EWL of rats in the heat because EWL as a function of time indicates a relatively constant rate of evaporation in the heat (Hainsworth and Stricker, 1970).

Following each experiment, the drying tubes were washed (Alconox, Alconox, Inc.), rinsed with distilled water, dried at 75.0° C (Stabil-Therm gravity oven, Blue M Electric Co.) and filled with fresh anhydrous calcium sulfate. Glass wool plugs were placed in the ends of each tube to prevent loss of calcium sulfate. Tubes were stored in

an air-tight vacuum dessicator until use, usually within 24 hr. The ends of each drying tube were wrapped with parafilm when not in use and no changes in tube weight were detected as the result of storage.

To measure the repeatability and effectiveness of the water vapor recovery technique, the rat was replaced in the container by its heat equivalent in the form of an electrical resistor (resistance = 100.5 Ohms). The resistor, in a beaker containing 100 ml of water, was connected to a power supply and, by applying 1.54 watts of power, the resultant water vapor released to the container simulated the EWL of a rat (Yuhaski, 1979). Evaporative water loss was measured, as described above, and compared to the volume of water lost from the beaker. From this a percent water recovery was calculated. This value, 0.961 ± 0.004 (mean ± S.E.), was obtained daily, before and after each experimental session. The daily average recovery error was used to correct all experimentally obtained EWL values.

The rate of airflow through the system was measured by flowmeters before and after each experiment (Figure 3). For each chamber, (1) airflow at the inlet was compared to airflow at the outlet and (2) airflow at the inlet was compared to airflow exiting from the complete system at the last calcium sulfate tube. No differences in inlet or outlet airflow rates were detected.

<u>Metabolic rate</u>. After the EWL measurements were completed, usually about 45 min following exposure to the experimental temperature, the rats remained in their respective containers and dry air passed through a tube containing anhydrous calcium sulfate and a flowmeter, at a constant rate of 500 ml/min, before entering

each container. Metabolic rate was measured according to the oxygen consumption method of Depocas and Hart (1957) as modified by Gwosdow-Cohen (1980). During the MR measurements, one container was vented to the room while the other was connected to the oxygen analyzer (Model 755, Beckman). From the exit port of one container the air passed through soda lime (Fisher Scientific) and calcium sulfate tubes to remove carbon dioxide and water vapor, respectively, before entering the oxygen analyzer (Figure 4). The latter was attached to a strip chart recorder (Model 110, Gould) and a graphic recording of oxygen consumption measurements over a 60 min period was obtained (Figure 5). The vented container was then attached to the oxygen analyzer for measurement of MR (ml oxygen consumed/min $\cdot$ $kg^{0.75}$) in the second rat.

Oxygen consumption ($\dot{V}O_2$) was calculated from the tracings (Figure 5) according to the method of Kelleher (1978):

$$\dot{V}O_2\ (ml/min \cdot kg^{0.75}) = \frac{\dot{V}_{air}}{BW^{0.75}} \times \frac{FO_{2in} - FO_{2out}}{100}$$

where, $\dot{V}_{air}$ is air flow rate in ml per min; BW is body mass in kilograms; FO_2 is the fraction of oxygen in the air entering (in) and exiting (out) the container; and 100 converts this fraction to a decimal. All volumes of consumed oxygen were corrected to standard conditions of temperature and pressure.

Statistics

Differences between treatments were determined by analysis of variance. Duncan's multiple range test was used to compare the means. Significance was assumed when $P < 0.05$.

Figure 4. Schematic of the apparatus used to measure metabolic rate.

Everything to the left of the dashed line was in the environmental room. For the sake of clarity, the manometer and thermometer are shown attached to only one container although pressure and temperature measurements were made in each container. (See text for details.)

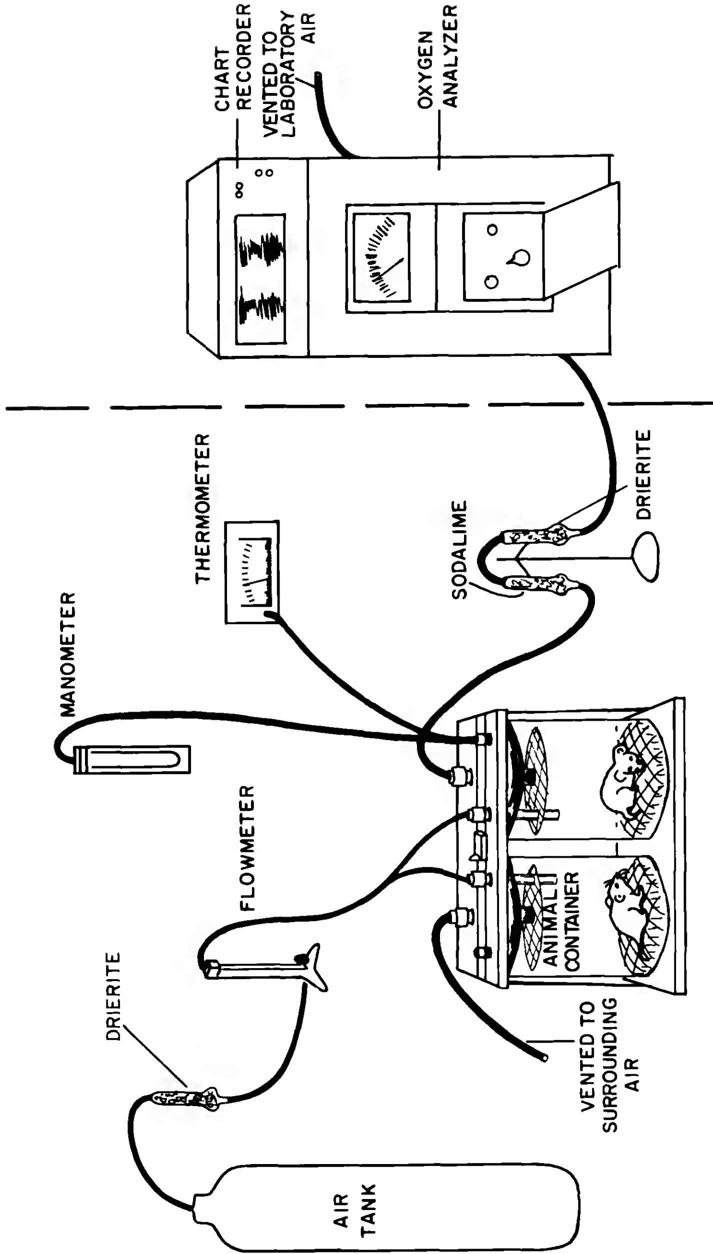


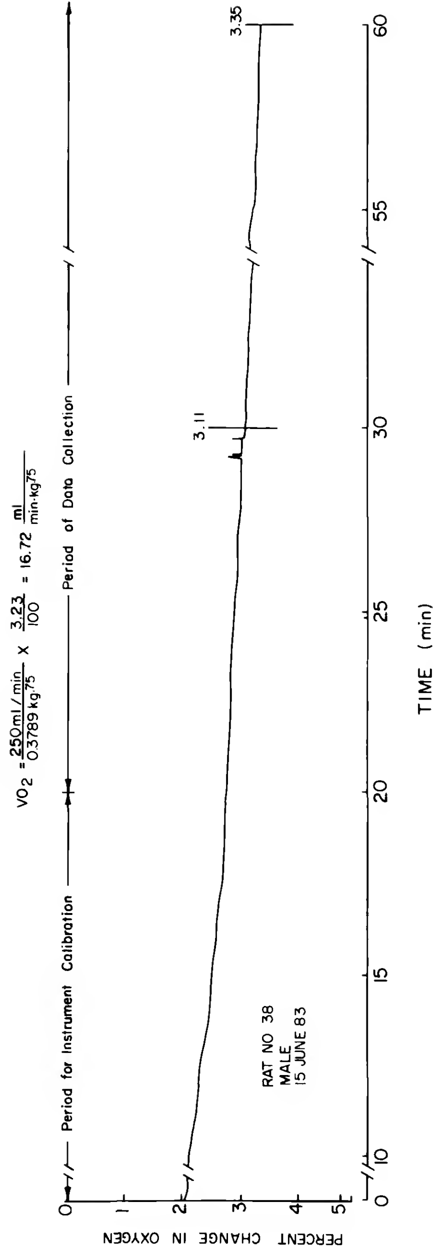
CHART RECORDER
VENTED TO LABORATORY AIR
OXYGEN ANALYZER
THERMOMETER
SODALIME
DRIERITE
MANOMETER
FLOWMETER
DRIERITE
ANIMAL CONTAINER
VENTED TO SURROUNDING AIR
AIR TANK

Figure 5. Typical record of oxygen consumption for rats.

In the usual case, FO_2 is measured at the beginning and end of the data collection period. The average of these numerical values is used in the equation for measurement of metabolic rate as ml oxygen consumed/min $\cdot$ $kg^{0.75}$:

$$\dot{V}O_2 = \frac{\dot{V}_{air}}{BW^{0.75}} \times \frac{FO_{2in} - FO_{2out}}{100}$$

where $\dot{V}_{air}$ is air flow in milliliters per minute; BW is body mass in kilograms; FO_2 is the fraction of oxygen in the air entering (in) and exiting (out) the container and 100 converts this fraction to a decimal. In this figure, the FO_2 values (3.11 and 3.35) are averaged and the ΔFO_2 is 3.23. The result of this sample calculation (16.72) has not been corrected for STPD.



VO2 = 250ml / min / 0.3789 kg.75 x 3.23 / 100 = 16.72 ml / min·kg.75
Period for Instrument Calibration
Period of Data Collection
RAT NO 38
MALE
15 JUNE 83
3.11
3.35
PERCENT CHANGE IN OXYGEN
0
1
2
3
4
5
TIME (min)
0
10
15
20
25
30
55
60

Results

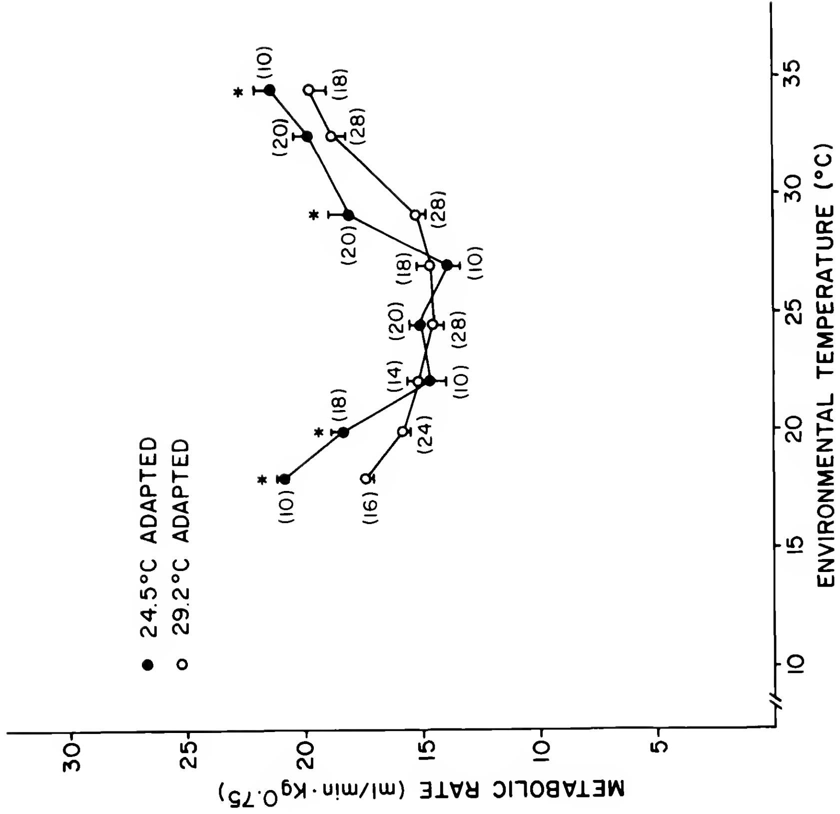
For rats adapted to the preconditioning temperature of 24.5° C, MR was relatively constant between experimental temperatures of 22.2 to 27.0° C, but significantly ($P < 0.05$) elevated at experimental temperatures of 20.0 and 29.2° C (Figure 6). Exposure to lower (18.0° C) and higher experimental temperatures (above 29.2° C) produced additional increases in MR. The MR of rats physiologically adapted to 29.2° C was relatively constant between experimental temperatures of 20.0 to 29.2° C (Figure 6) but significantly elevated following acute exposure to experimental temperatures above and below this range.

Rectal and tail skin temperatures varied with preconditioning temperature. Rats adapted to 29.2° C maintained highly significant ($P < 0.01$) elevations in Tr (37.0 ± 0.05, mean ± S.E., n = 190) compared to rats (36.5 ± 0.07, n = 117) adapted to 24.5° C. Tail skin temperatures produced similar results: 29.2° C adapted rats maintained highly significant elevations in Tts (32.1 ± 0.10, n = 50) compared to 24.5° C adapted rats (28.5 ± 0.13, n = 50).

Minimal rectal temperature differentials (ΔTr) for rats adapted to 24.5° C were observed between 20 to 24.5° C (Figure 7), a range that is similar to that for relatively constant MR (Figure 6). These rats had significantly reduced ΔTr at an experimental temperature of 18.0° C and a significantly increased ΔTr at temperatures above 29.2° C. Compared to 24.5° C adapted rats, the 29.2° C adapted group displayed a reduced but relatively constant ΔTr between 20.0 and 27.0° C, a range that is quantitatively similar to that for relatively constant MR (Figure 6).

Figure 6. Metabolic rate (oxygen consumption) of rats preconditioned to different environmental temperatures (24.5 or 29.2° C) and exposed to experimental temperatures between 18.0 and 34.5° C for 3 hr.

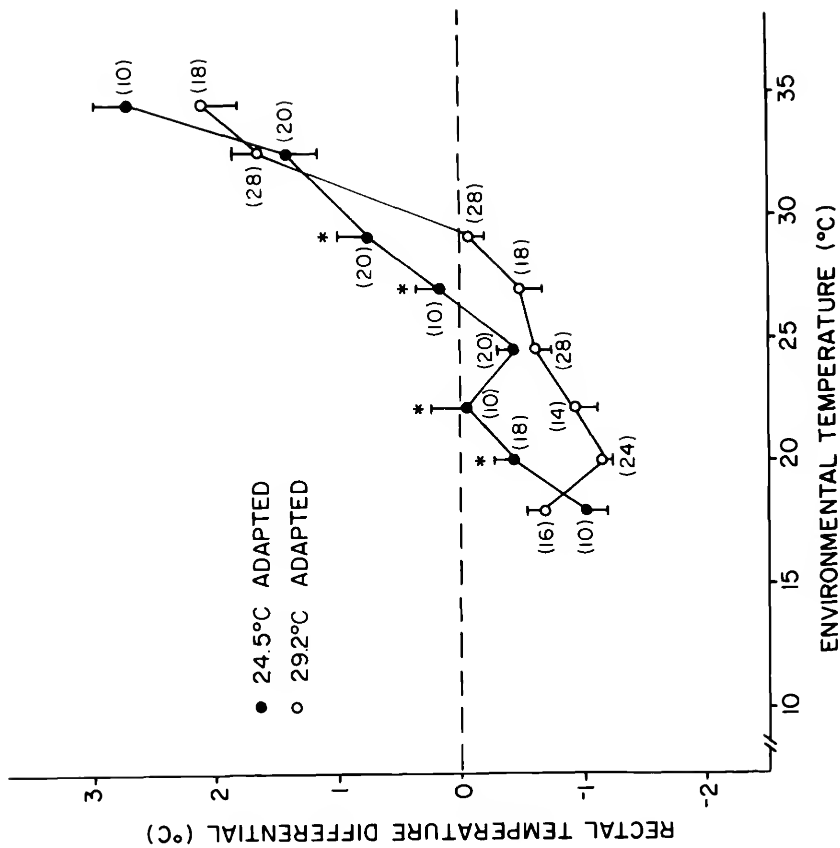
Each point represents the mean value and the vertical bars the standard errors. Asterisks indicate significant ($P < 0.05$) differences between groups and values in parentheses indicate the number of rats used.



• 24.5°C ADAPTED
○ 29.2°C ADAPTED
METABOLIC RATE (ml/min·Kg$^{0.75}$)
ENVIRONMENTAL TEMPERATURE (°C)
30
25
20
15
10
5
10
15
20
25
30
35
(10)
(16)
(18)
(24)
(14)
(10)
(20)
(28)
(18)
(10)
(20)
(28)
(20)
(28)
(10)
(18)
*

Figure 7. Rectal temperature differential of rats preconditioned to different environmental temperatures (24.5 or 29.2° C) and exposed to experimental temperatures between 18.0 and 34.5° C for 3 hr.

Each point represents the mean value and the vertical bars the standard errors. Asterisks indicate significant ($P < 0.05$) differences between groups and the values in parentheses indicate the number of rats used.



RECTAL TEMPERATURE DIFFERENTIAL (°C)
3
2
1
0
-1
-2
ENVIRONMENTAL TEMPERATURE (°C)
10
15
20
25
30
35
● 24.5°C ADAPTED
○ 29.2°C ADAPTED
(10)
(16)
(18)
(24)
(10)
(14)
(20)
(28)
(10)
(18)
(20)
(28)
(28)
(20)
(10)
(18)
*

At 32.5° C, the ΔTr of the 29.2° C adapted animals was significantly increased, but no further elevation was observed at 34.5° C.

Changes in Tts were more variable than in ΔTr. Rats adapted to 24.5° C displayed significantly decreased tail skin temperature differentials (ΔTts) when acutely exposed to 20.0° C and 18.0° C compared to 24.5° C (Figure 8). On the other hand, a significantly elevated ΔTts occurred at 29.2° C and 32.5° C. Although ΔTts followed a similar pattern for 29.2° C adapted rats, these changes were greater in magnitude than for the 24.5° C adapted rats. The 29.2° C adapted group maintained significantly lower ΔTts than 24.5° C adapted rats at all experimental temperatures.

Evaporative water loss differentials (ΔEWL) for both groups of rats were unchanged between 18.0 and 27.0° C (Figure 9). However, the 24.5° C adapted group maintained a consistently elevated ΔEWL ($P < 0.05$) compared to the 29.2° C adapted group. Rats adapted to 24.5° C displayed significantly elevated ΔEWL at temperatures of 29.2° C or higher whereas the ΔEWL for the 29.2° C adapted group was significantly elevated at temperatures of 32.5° C or above.

Estimates of heat loss through evaporation indicate that evaporation accounts for a great amount of the metabolic heat production as environmental temperatures are raised (Table 2). Rats adapted to 29.2° C dissipated more heat at experimental temperatures above 32.5° C compared to 24.5° C adapted rats; at 34.5° C these rats lost 20% more heat by evaporation than their 24.5° C adapted counterparts.

The relative food intake of 24.5° C adapted rats were greater ($P < 0.05$) than that of the 29.2° C group (Table 3). The latter group

Figure 8. Tail skin temperature differential of rats preconditioned to different environmental temperatures (24.5 or 29.2° C) and exposed to experimental temperatures between 18.0 and 32.5° C for 3 hr.

Each point represents the mean value and the vertical bars the standard errors for 10 rats. Asterisks indicate significant ($P < 0.05$) differences between groups.

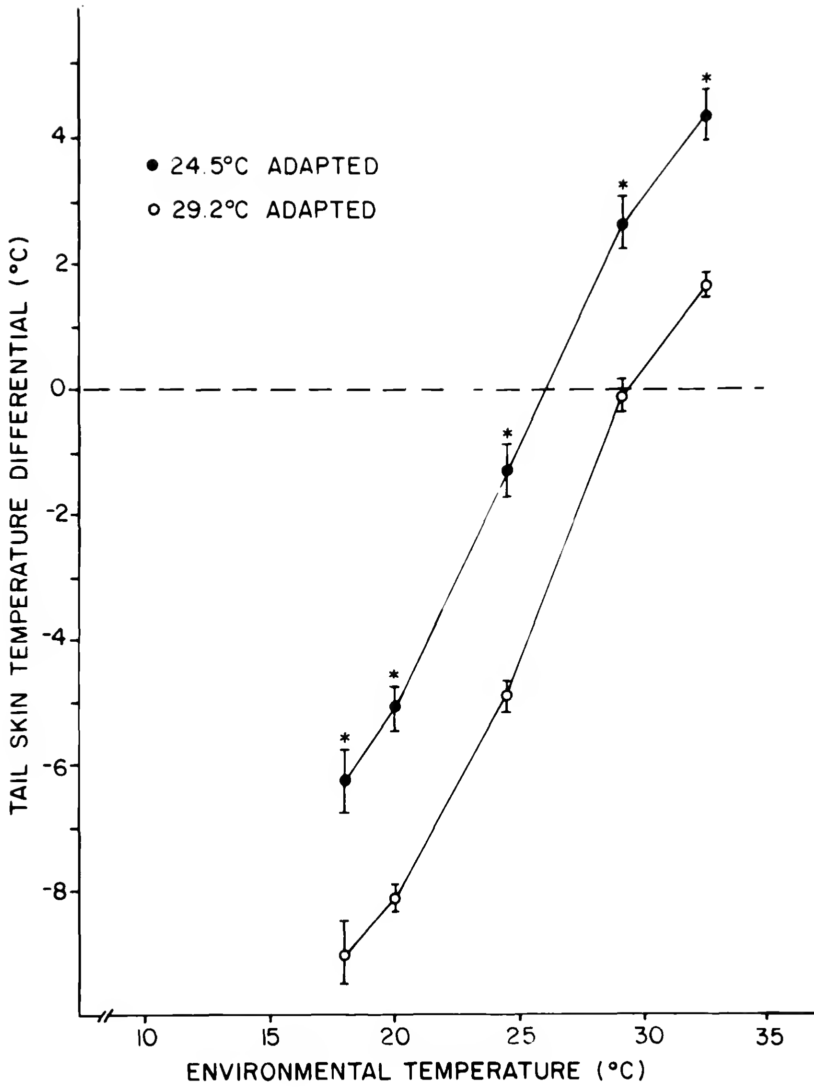
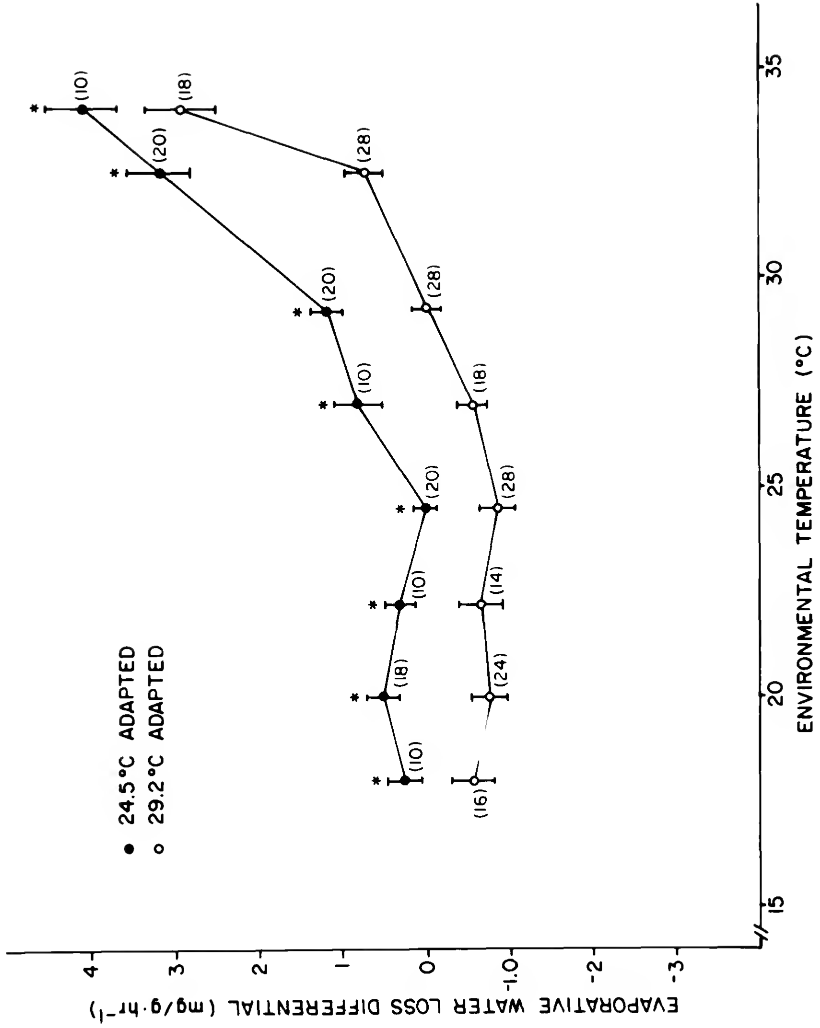

24.5°C ADAPTED
29.2°C ADAPTED
TAIL SKIN TEMPERATURE DIFFERENTIAL (°C)
4
2
0
-2
-4
-6
-8
10
15
20
25
30
35
ENVIRONMENTAL TEMPERATURE (°C)

Figure 9. Evaporative water loss differential of rats preconditioned to different environmental temperatures (24.5 or 29.2° C) and exposed to experimental temperatures between 18.0 and 34.5° C for 1 hr.

Each point represents the mean value and the vertical bars the standard errors. Asterisks indicate significant ($P < 0.05$) differences between groups and the values in parentheses indicate the number of rats used.



24.5 °C ADAPTED
29.2 °C ADAPTED
EVAPORATIVE WATER LOSS DIFFERENTIAL (mg/g·hr-1)
ENVIRONMENTAL TEMPERATURE (°C)
4
3
2
1
0
-1.0
-2
-3
15
20
25
30
35

Table 2. Heat production and evaporative heat loss as a function of environmental temperature.

Temperature (° C)		Metabolic rate*		Evaporative water loss		% heat produced lost by evaporation
Precondition	Experimental	$ml/g \cdot hr^{-1}$	$cal/g \cdot hr^{-1}$	$mg/g \cdot hr^{-1}$	$cal/g \cdot hr^{-1}$	
24.5	24.5	1.07	5.16	1.72	1.00	19.38
24.5	32.5	1.42	6.84	2.86	1.66	24.27
24.5	34.5	1.62	7.82	4.60	2.67	34.14
29.2	29.2	1.11	5.35	1.97	1.14	21.31
29.2	32.5	1.20	5.78	2.97	1.72	29.76
29.2	34.5	1.26	6.07	5.61	3.25	53.54

* The caloric value = 4.82 calories per ml of oxygen consumed (Ganong, 1977).

Table 3. Relative food and water intake and body mass of rats preconditioned to different environmental temperatures.

Group	Daily food intake* (g/100 g body wt)	Daily water intake** (g/100 g body wt)	Body mass** (g)
24.5° C adapted	$6.6 \pm 0.07^{a***}$	11.2 ± 0.38^{a}	385.48 ± 6.24^{a}
29.2° C adapted	5.5 ± 0.10^{b}	12.7 ± 0.40^{b}	447.66 ± 10.90^{b}

* All values are expressed as mean ± S.E. for 26 rats adapted to 24.5° C and 34 rats preconditioned to 29.2° C for 24 days.

** Initial body mass for 24.5° C adapted and 29.2° C adapted rats after the two week preconditioning period.

*** Different superscripts (a-b) within each column differ significantly ($P < 0.05$) as determined by analysis of variance.

consumed ($P < 0.05$) more water than 24.5° C rats. In general, rats adapted to 29.2° C were larger than 24.5° C adapted rats.

Discussion

The present research extends previous findings on thermoneutral zones (TNZ) for rats and suggests that these zones depend upon the thermal history of the rat. For example, rats adapted to 24.5° C displayed a TNZ of about 22.2° C to 27.0° C, while their 29.2° C counterparts had a TNZ of about 20.0° C to 29.2° C (Figure 6). Within these temperature ranges the rats maintained a relatively constant ΔTr and MR.

The increased metabolic heat production of 24.5° C adapted rats resulted in relatively constant ΔTr when exposed to cool (20.0° C) temperatures and contributed to an increased ΔTr at 29.2° C or above. At these higher temperatures the elevated MR of 24.5° C adapted rats were parallel to increases in ΔTr (Figure 7). Rats adapted to 29.2° C maintained a ΔTr below their preconditioning temperature and this may be due to decreased thyroid or adrenal gland activity or thinned fur coat. Such physiological adaptations ensure survival at elevated temperatures by reducing metabolic heat production and facilitating heat loss (Collins and Weiner, 1968).

At their respective preconditioned temperatures, the absolute Tr and Tts of 29.2° C adapted rats were maintained at an elevated level compared to 24.5° C adapted rats. The resultant increased temperature gradient between the rat and the environment may have facilitated heat loss when the animals were exposed to the various experimental temperatures. Maintenance of Tr at a constant new level 0.5-1.0° C

above control values indicates physiological adaptation to the heat (Horowitz, 1976) and suggests an increased reference point for regulation of body temperature.

By regulating the blood flow to the tail, rats may conserve or dissipate heat (Grant, 1963; Rand et al., 1965; Stricker and Hainsworth, 1971; Hellstrom, 1975; Young and Dawson, 1982). In the present experiment, Tts is used as an index of heat conservation (vasoconstriction) or heat dissipation (vasodilation). Generally, tail vasoconstriction was evident at the lower temperatures (18.0 to 20.0° C) and tail vasodilation at elevated environmental temperatures (32.5° C or above) for both groups of rats (Figure 8). Both the magnitude of ΔTts and the experimental temperatures at which vasoconstriction and vasodilation occurred varied with preconditioning temperature. Rand et al. (1965) and Thompson and Stevenson (1966) also found the ambient temperature causing vasodilation of the rat tail varied with acclimatization temperature. These changes may be due to a modified sensitivity of peripheral (Rand et al., 1965) or central (Dawson and Keeber, 1979) thermal receptors.

As environmental temperatures increase, the temperature gradient between the rat and the environment decreases and EWL becomes an important heat loss mechanism (Hainsworth, 1968). By increasing Tr in the heat, the rats widen the temperature gradient between themselves and the environment which facilitates heat loss by conduction, convection and radiation. Because heat loss by these means is not sufficient for body temperature regulation in the heat (Stricker and Hainsworth, 1970), rats must cool themselves by evaporating saliva which is groomed onto their body surfaces (Hainsworth et al., 1968). Both groups of

rats in the current experiment increased ΔTr to similar values and the ΔTts of 24.5° C adapted rats was higher ($P < 0.05$) than the 29.2° C adapted group, indicating a greater loss of heat through the tail for the former group. At 32.5° C, the 24.5° C adapted rats also maintained an elevated EWL compared to the 29.2° C adapted rats.

Swift and Forbes (1939) have shown that 25° C adapted rats lost 49.2% of their body heat by evaporation at 33° C. Studies by Hainsworth and Stricker (1971) produced similar results. In the experiment reported herein, rats adapted to 24.5° C lost 24.3% while 29.2° C adapted rats lost 29.8% of their body heat by evaporation in the heat (32.5° C). At 34.5° C these values are 34.1% and 53.5% for 24.5° C and 29.2° C adapted rats, respectively (Table 2).

Because water is the main source of cooling for the body under elevated temperatures, physiological mechanisms operate to replace water lost by evaporative cooling (Hainsworth, 1968). In this study, water was available _ad libitum_ and significant changes in water intake (Table 3) were observed on exposure to heat. The increased water intake may have compensated for the water lost by evaporation. Although increased drinking by rats in the heat has been shown to be secondary to dehydration (Hainsworth et al., 1968), water intake also may be triggered by the stimulation of peripheral or central thermoreceptors (Andersson and Larson, 1961) and drying of the oropharyngeal mucous membranes (Gregerson and Cannon, 1932; Lund et al., 1969).

As environmental temperatures increased, the appearances and behavior of the rats were indicative of the rat's ability to thermoregulate as reported by Stricker et al. (1968). All rats

appeared to have similar behavioral adjustments after placement in the EWL chamber for about 10 min. The rats settled into a prone resting position and, except for periodic adjustments and grooming behavior, remained quiet for the duration of the experimental session. Initially the rats groomed the face and neck, but as ambient temperatures increased, the scrotum and tail were emphasized. Eventually, the entire ventral surface was covered with saliva and the animals were "soaking wet."

The decreased food intake observed in rats preconditioned to 29.2° C for at least 2 weeks agrees with previous reports by Attah and Besch (1977) and Gwosdow-Cohen (1980). Brobeck (1960) and Hamilton (1963) also have reported decreased food intake at high ambient temperatures. The reduced food intake may indicate a lower energy requirement which, in turn, may reflect changes in metabolic activity in the heat (Cassuto, 1968). Efficiency of food conversion is increased in the heat, so the decreased food intake may be utilized more efficiently (Pennycuik, 1964). This may account for some of the body mass differences between 24.5° C and 29.2° C adapted rats. Attah and Besch (1977) reported that rats gained weight in the heat despite significantly reduced food intake. Also, rats at high environmental temperatures are less active (Howard et al., 1959). These changes contribute to the decreased internal heat production of heat adapted rats (Brobeck, 1960). Such control of food intake has been reported by Collins and Weiner (1968) to be crucial in counteracting hyperthermia.

At their respective preconditioned temperatures, the absolute rectal and tail skin temperatures of 29.2° C adapted rats were maintained at an elevated level compared to 24.5° C adapted rats.

At temperatures above 29.2° C changes in MR, Tts and EWL all were of a greater magnitude for the 24.5° C adapted rats compared to the 29.2° C rats but similar ΔTr were observed in both groups. Because exposure of 29.2° C adapted rats to temperatures below 29.2° C produced greater changes than for 24.5° C adapted rats, it seems likely that these two groups were regulating their thermal responses around different reference points. Moreover, the different upper critical temperatures of the thermoneutral zones of both groups also indicate a changed sensitivity to heat. Thermoregulatory responses are regulated around a "set-point" temperature (Adolph, 1964; Folk, 1974), and the altered "heat sense" of these two groups of rats could indicate that the preconditioning temperatures in this study shifted this reference point for regulation of body temperature.

Based on these results, future studies on physiological responses of rats to heat should take into consideration the rat's thermal history. This would enable a comparison of data from animals exposed to temperatures within the range of 22.2 to 27.0° C (control temperature). Moreover, it is necessary that control temperatures be precisely reported so that the responses of rats may be compared to similar experiments from other laboratories.

Summary

After preconditioning individually housed male Sprague-Dawley rats to temperatures of either 24.5° C or 29.2° C for 14 days, randomly paired animals from each group were acutely exposed (3 hr), in series, to experimental temperatures between 18.0° C and 34.5° C in a controlled environment room. Relative humidity of 50 ± 0.3% and

a 12L:12D photoperiod (L = 0900 to 2100 hr) were maintained for all experiments. Metabolic rate (MR) and evaporative water loss (EWL) were measured using an openflow system; rectal (Tr) and tail skin (Tts) temperatures using thermistors. For rats physiologically adapted to 24.5° C, MR was relatively constant over a temperature range of 22.2 to 27.0° C; for 29.2° C rats, over a range of 20.0 to 29.2° C. Above and below these ranges, MR for both groups was significantly ($P < 0.05$) elevated. Between 20.0 to 29.2° C, Tr changes for 24.5° C adapted rats were significantly ($P < 0.05$) different from 29.2° C rats; similar changes were observed in Tts. Although EWL for both groups was relatively constant between 18.0 to 27.0° C, 24.5° C adapted rats displayed consistently higher EWL changes at all environmental temperatures above 27.0° C. These data suggest that preconditioning temperatures may alter the reference point around which body temperature is regulated.

CHAPTER 2
BODY TEMPERATURE RESPONSES INDUCED BY BETA-ENDORPHIN IN RATS AT 24.5° C

Introduction

The data presented in Chapter 1 suggest that preconditioning temperatures alter thermoregulatory responses in the rat which, in turn, change the reference point around which body temperature is regulated. It was previously reported that "set-point" temperatures also may be modified by opioids (Holaday et al., 1980; Clark, 1981); these body temperature alterations appear to be influenced by dose and route of opioid injection (Yehuda and Kastin, 1980).

It has been reported that both physiological levels and low doses of beta-endorphin (B-END) produce hyperthermia (Blasig et al., 1979), but high doses of B-END produce either hypothermia or hyperthermia followed by a hypothermic (biphasic) response (Clark, 1981). Although B-END does not readily cross the blood-brain barrier (Reilly et al., 1980), intravenous (IV) administration produces a slight hyperthermia in rats at room temperature (Yehuda et al., 1980). Intracerebroventricular (IVT) injections of B-END cause both thermal and analgesic responses which are dependent on the administered dose (Yehuda and Kastin, 1980).

Naloxone has been reported to block (Holaday et al., 1978a; Clark, 1981), or antagonize (Brown et al., 1977; Blasig et al., 1978) the body temperature response to B-END. In some experiments, naloxone

has had no effect on body temperature (Bloom and Tseng, 1979). Bloom and Tseng (1981) reported naloxone antagonism of opioid-induced hypothermia but not hyperthermia.

Although other substances (e.g., prostaglandin E_2) may produce body temperature changes similar to those previously observed for B-END (Yehuda and Kastin, 1980), no studies dealing with alterations in body temperature by leucine-enkephalin and angiotensin II in rats have been reported. Leucine-enkephalin is similar in structure to B-END while angiotensin II possesses a molecular weight similar to B-END.

The present study was conducted to clarify the dose-response relationship between B-END and body temperature in the rat and to compare the specificity of action of B-END, leucine-enkephalin (opioid) and angiotensin II (non-opioid) on body temperature in rats preconditioned to a thermoneutral temperature of 24.5° C. The role of calorigenic hormones from the adrenal and thyroid glands also was examined. The results are reported here.

Materials and Methods

Animals

Adult (2 months old), male Sprague-Dawley rats [Caw: CFE (SD)] were used in these experiments. The rats (350.5 ± 6.2 g, mean ± S.E.) were housed individually in metabolic cages (Model 4-641-000, Acme) in a controlled environment room (Model C7-88, Forma Scientific) at 24.5 ± 0.1° C, 50 ± 0.3% relative humidity and a 12L:12D photoperiod (L = 0900 to 2100 hr) for at least 2 weeks before experiments began. Water bottles equipped with sipper tubes were attached to each cage.

Food (Laboratory Rodent Chow, Ralston Purina Company) and water were available *ad libitum*.

Environmental Room

The ambient temperature in the environmental room was monitored as described in Chapter 1.

Drugs

Varying doses of B-END (0.05-50 μg), leucine-enkephalin (1-50 μg) and angiotensin II (0.01-1 μg) were prepared in sterile physiological saline. The injection volumes for all substances (Beckman) were 5 μl and 0.3 ml for IVT and IV routes of administration, respectively. Naloxone (Endo Laboratories), propranolol (Sigma Chemicals, Inc.) and phentolamine (Ciba-Geigy Pharmaceuticals) were injected intraperitoneally (IP). Ampicillin (Bristol Laboratories) and procaine penicillin-G (Pfizer) were used post-operatively to prevent infection. Antibiotics were discontinued 48 hr before beginning experiments and caused no observed rectal temperature (Tr) changes. A series of control rats received similar treatment.

Experimental Procedures

General. Experiments were conducted on unrestrained rats placed in standard rodent shoebox cages in a controlled environment room. After a 30 min control period, rats were injected IVT and briefly removed from the cages, at 0.5 hr intervals, for measurement of rectal temperature over 3 hr. An additional Tr measurement was recorded 75 min post-injection for rats injected with naloxone. The Tr of rats

pretreated with propranolol and phentolamine were measured every 15 min for the first hour followed by 30 min measurements for 2 additional hr.

Intracerebroventricular cannulae. Intracerebroventricular injections were through stainless steel cannulae implanted into the right lateral ventricle while each rat was under ether anesthesia. Cannula placement was determined by the de Groot system as modified by Pellegrino et al. (1979) at least 72 hr before experiments began. Correct positioning of each cannula was verified initially by the backward flow of cerebrospinal fluid. Further verification was obtained at necropsy and by cerebral ventriculography (Appendix C).

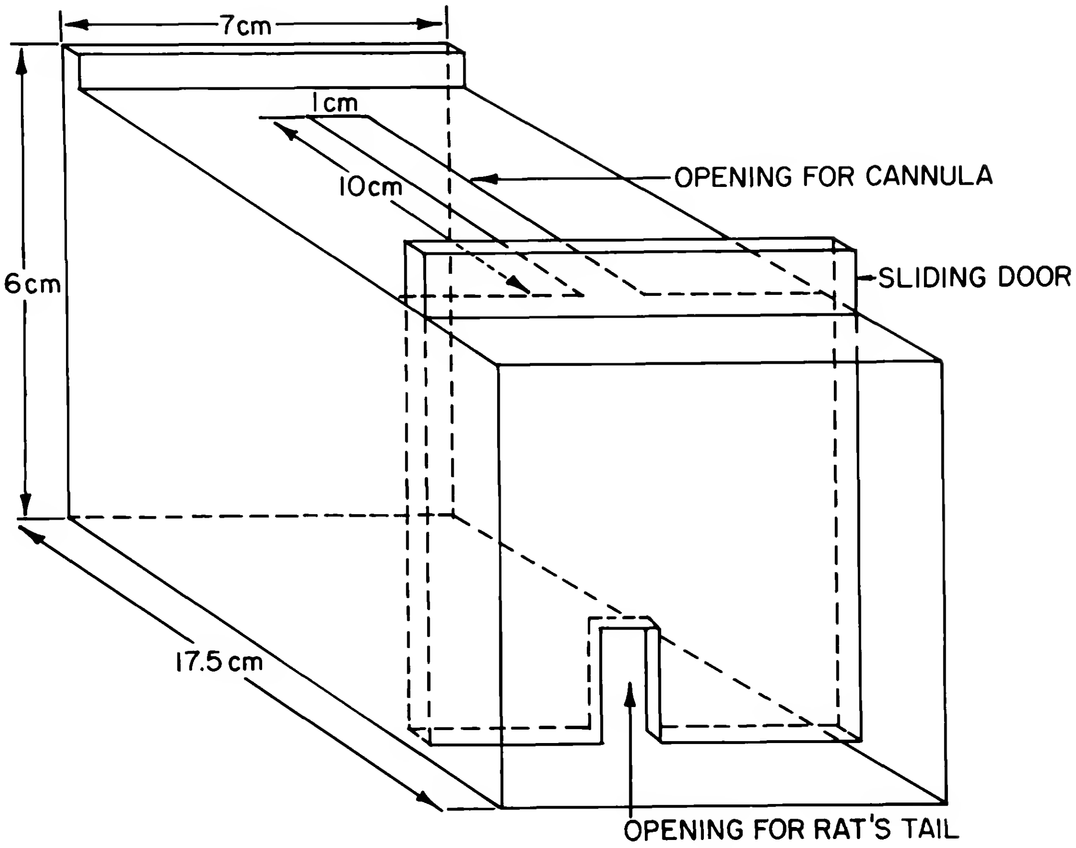
Intravenous cannulae. Under ether anesthesia, a permanent intravenous (IV) cannula was implanted into the right jugular vein of each rat, as detailed by Popovic et al. (1963). A 24 hr recovery period was allowed before beginning experiments. Patency of the cannula was maintained by flushing daily with 0.1 ml of heparinized (Lypo-Med, Inc.) saline solution (1000 units/ml).

Experiments requiring IV injections or blood sampling were conducted in an acrylic plastic (Plexiglas) sampling box 7 cm wide, 17.5 cm long and 6 cm high (Figure 10). The top of the sampling box was equipped with a 10 cm long and 1 cm wide opening for easy access to the IV cannula. A sliding door with an opening for the rat's tail allowed easy access but assured containment of the rat. The box allowed free movement of the rat.

Adrenalectomized rats. Adrenalectomy was performed under ether anesthesia through the bilateral paralumbar approach (Zarrow

Figure 10. Diagrammatic sketch of the sampling box used for intravenous injections and blood sampling.

(See text for details.)


7cm
6cm
1cm
10cm
OPENING FOR CANNULA
SLIDING DOOR
17.5 cm
OPENING FOR RAT'S TAIL

et al., 1964). Following surgery, rats were maintained on a 0.9% NaCl drinking solution, available *ad libitum*. Completeness of adrenalectomy was verified at necropsy and by serum corticosterone levels (Gwosdow-Cohen et al., 1982, Appendix B). A 1 week recovery period was allowed before beginning experiments.

Hypophysectomized rats. Hypophysectomized rats (Charles River Breeding Laboratories) were maintained on a 0.5% dextrose drinking solution, available *ad libitum*. Hypophysectomy was verified by lack of change in body mass. A 2 week recovery period was allowed before experiments began.

Radioimmunoassays (RIA). Serum corticosterone levels were measured according to the procedure (Appendix B) detailed by Gwosdow-Cohen et al. (1982). Measurements of serum thyroid hormones were made using commercially available RIA kits which appear to be appropriate for determining these hormones in rat serum (Clinical Assays).

Measurements

All measurements or sample collection were performed between 1500 and 1800 hr.

Body temperature. The procedure for measurement of rectal temperature was described in Chapter 1.

Statistical Analysis

Differences between treatments were determined by analysis of variance. Duncan's multiple range test was used to compare the means.

Analysis of variance, log transformation and linear regression were used to determine dose-response relationships. Significance was assumed when $P < 0.05$.

Results

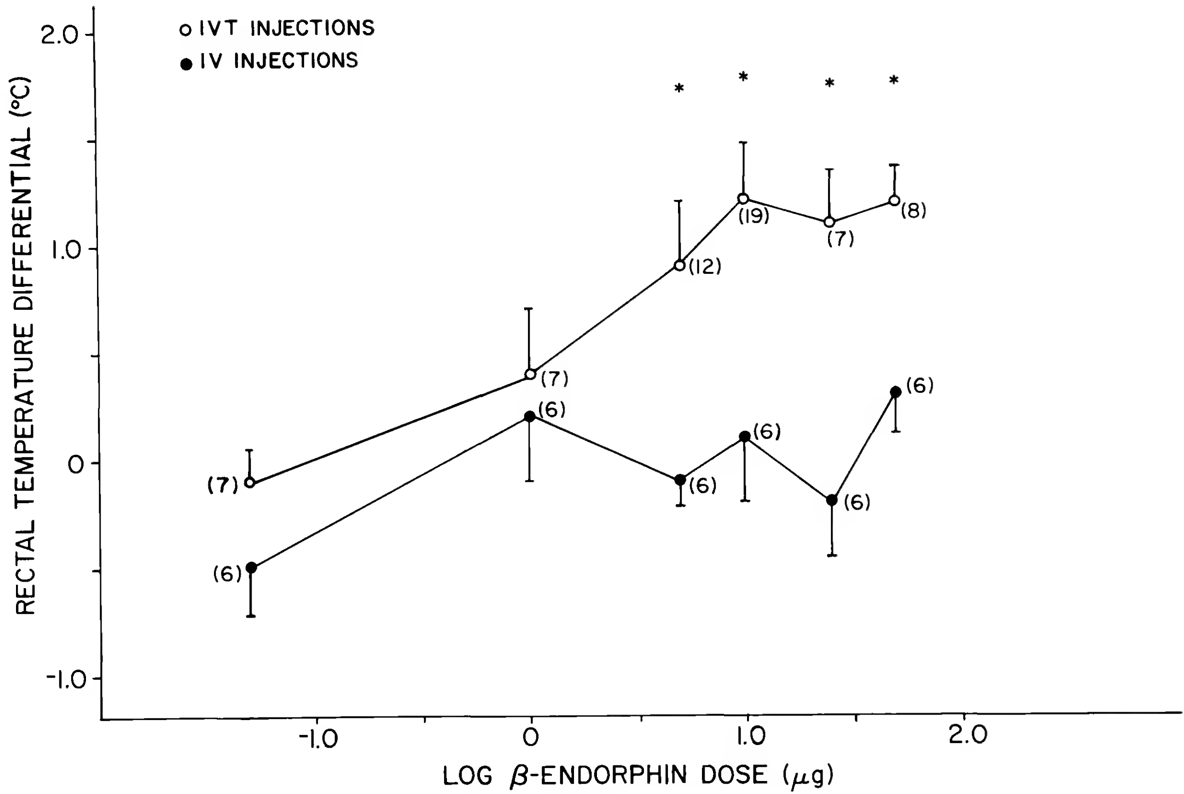
Intracerebroventricular injections of varying doses (5-50 μg) of B-END resulted in increased rectal temperature differentials (ΔTr) within 30 min post-injection. Rectal temperature remained elevated for at least 90 min, then declined and returned to control values of saline-treated rats within 180 min post-injection. Because the maximal Tr response occurred 60 min post-injection, this time interval was used in all subsequent measurements.

Following IV administration of B-END in doses between 0.05 and 50.0 g, no significant differences in ΔTr were observed (Figure 11). However, for rats injected IVT with B-END, the ΔTr was highly significantly ($P < 0.01$) elevated above the ΔTr of IV administered rats at all doses between 5.0 and 50 μg. On the other hand, IVT injections of varying doses of leucine-enkephalin (1-50 μg) and angiotensin II (0.01-1.0 μg) did not alter ΔTr (Table 4).

A significant relationship between ΔTr and doses of B-END between 0.05 and 50 μg was observed during the 60 min post-IVT injection time interval (Figure 12). The dose-response curve (Figure 12) was plotted as ΔTr vs. the log dose of B-END and calculated using analysis of variance and linear regression. Verification of the results were obtained using the log transformation (Alder and Roessler, 1964). Correctness of fit for both statistical models was highly significant

Figure 11. Comparison of intravenous (IV) and intracerebroventricular (IVT) injections of varying doses of beta-endorphin on body temperature changes 60 min post-injection.

Each point represents the mean value and the vertical bars the standard errors. Asterisks indicate significant ($P < 0.05$) differences between groups and the values in parentheses indicate the number of rats used.



○ IVT INJECTIONS
● IV INJECTIONS
RECTAL TEMPERATURE DIFFERENTIAL (°C)
2.0
1.0
0
-1.0
LOG β-ENDORPHIN DOSE (μg)
-1.0
0
1.0
2.0
(7)
(6)
(7)
(6)
(12)
(6)
(19)
(6)
(7)
(6)
(8)
(6)
*
*
*
*

Table 4. Rectal temperature differentials in rats at 24.5° C 60 min post-intracerebroventricular injection of angiotensin II and leucine-enkephalin.

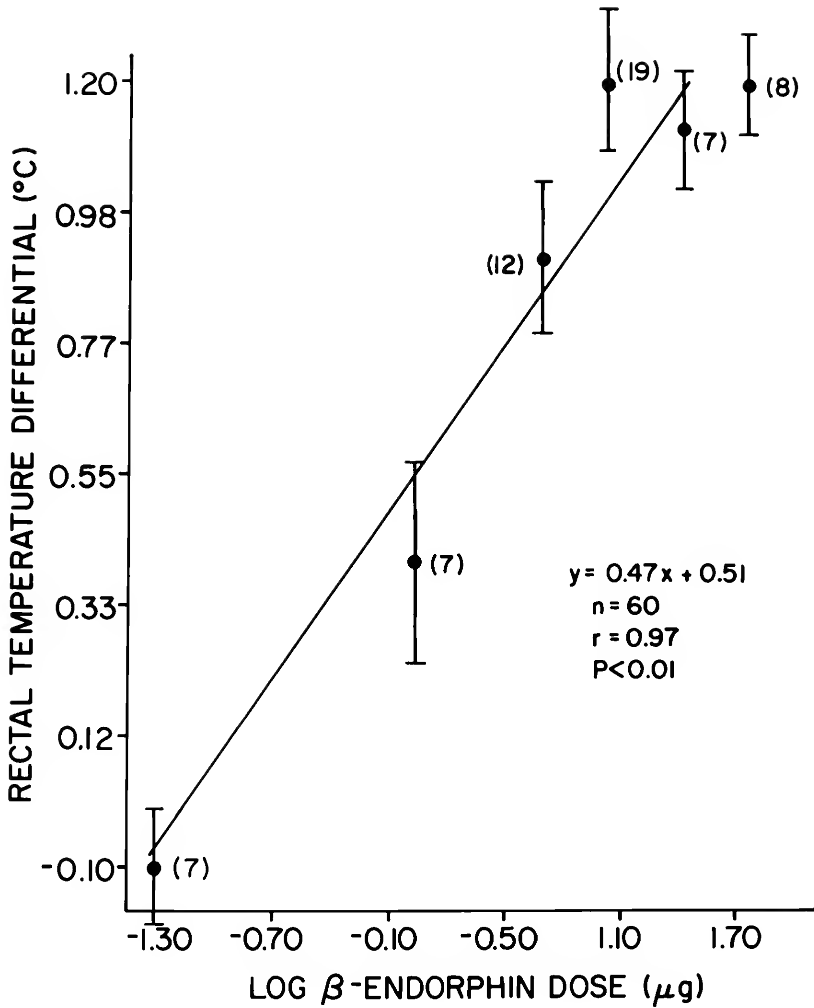
Dose (μg)	Rectal temperature differential* (° C)			
	n**	Angiotensin II	n	Leucine-Enkephalin
0.01	7	-0.1 ± 0.21		
0.10	6	0.2 ± 0.19		
1.0	7	0.1 ± 0.36	6	0.2 ± 0.21
10.0			6	0.7 ± 0.27
50.0			6	0.0 ± 0.26

* Rectal temperature differential = rectal temperature 60 min post-injection minus rectal temperature 0 time, corrected for saline value and expressed as mean ± S.E.

** Number of rats.

Figure 12. The relationship between dose of beta-endorphin and change in rectal temperature in the rat.

Each point represents the mean value and the vertical bars the standard errors. Values in parentheses indicate the number of rats used.


RECTAL TEMPERATURE DIFFERENTIAL (°C)
1.20
0.98
0.77
0.55
0.33
0.12
-0.10
(19)
(8)
(7)
(12)
(7)
(7)
y= 0.47x + 0.51
n = 60
r = 0.97
P<0.01
-1.30
-0.70
-0.10
-0.50
1.10
1.70
LOG β-ENDORPHIN DOSE (μg)

($P < 0.01$). The data (Figure 12) is represented by the simpler statistical model.

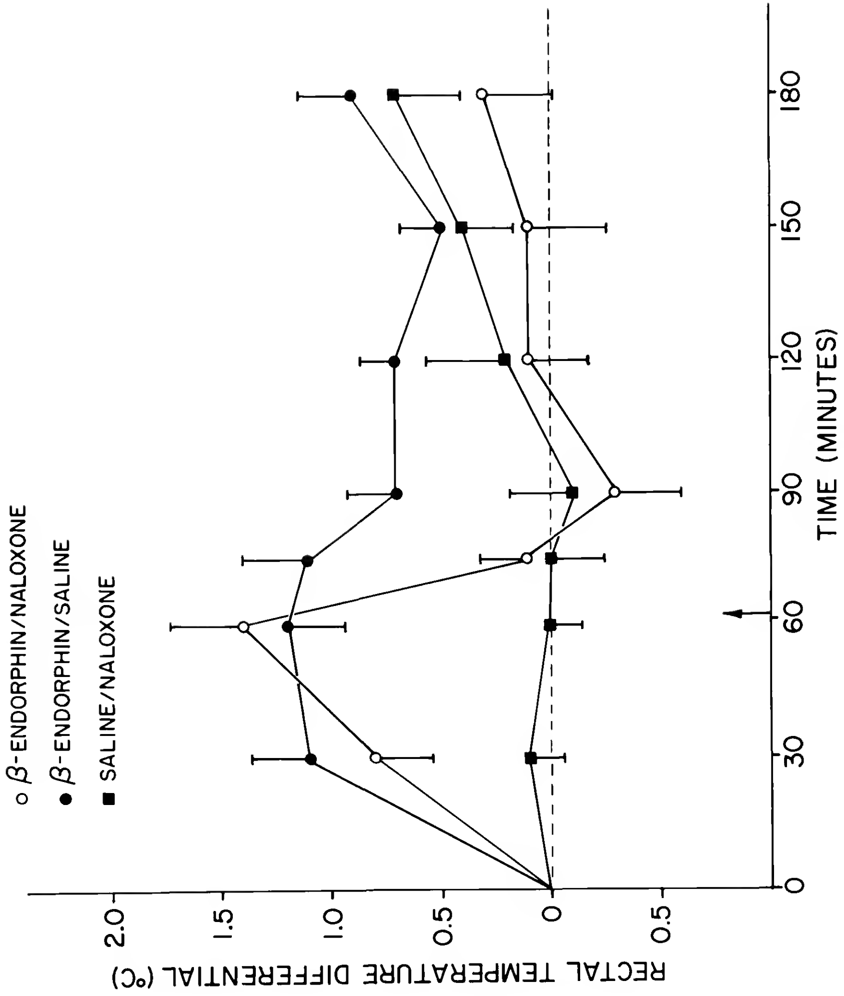
The hyperthermia produced by IVT injections of B-END was antagonized by naloxone injected IP (Figure 13). This response was rapid, and ΔTr returned to control levels within 15 min following naloxone injection. Administration of saline did not alter the hyperthermia resulting from IVT injected B-END. Rats receiving IVT saline followed by naloxone did not display significant changes in ΔTr.

Adrenalectomized and hypophysectomized rats receiving varying doses (between 0.05 and 50.0 μg) of IVT injected B-END generally displayed an increased ΔTr, but not to the same level as intact rats (Figure 14). Beta-endorphin doses between 10 and 50 μg induced highly significant decreases in ΔTr of adrenalectomized compared to intact rats. For hypophysectomized rats, lowered ($P < 0.01$) ΔTr were observed only at doses of 10 and 50 μg of B-END. Moreover, a dose-response relationship similar to that demonstrated with intact rats (Figure 12) could not be obtained with either adrenalectomized or hypophysectomized rats. Dose-related changes in serum corticosterone (Table 5) or thyroid hormone differentials (Table 6) could not be detected in rats receiving IVT injections of varying doses of B-END. However, serum T_3 levels (absolute and differential values) were significantly reduced below saline (zero baseline) levels at all doses of B-END administered.

Intraperitoneal injections of propranolol (6 mg/kg IP) lowered, although not significantly, ΔTr compared to saline controls (Figure 15). Rats administered phentolamine (6 mg/kg IP) significantly decreased ΔTr compared to saline-treated animals. The ΔTr effect

Figure 13. Time course effects on rectal temperature of beta-endorphin followed by saline (●), beta-endorphin followed by naloxone (o) and saline followed by naloxone (■) on rectal temperature differential for rats at 24.5° C.

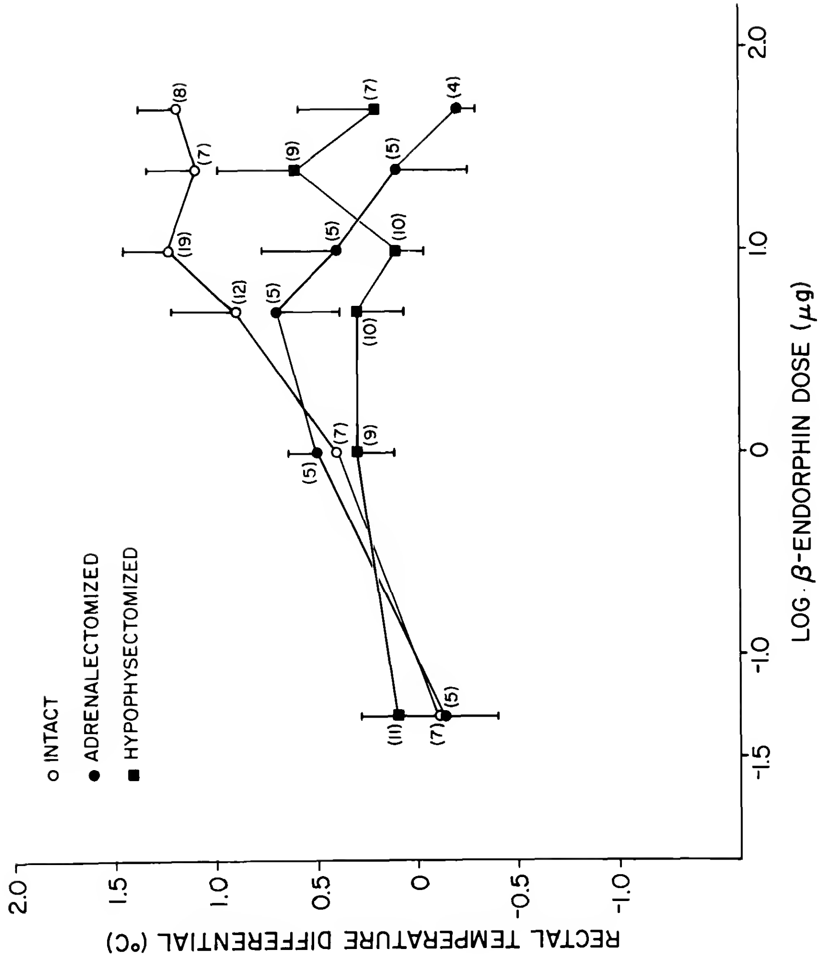
Intracerebroventricular injection of beta-endorphin (10 μg) or saline was made at 0 time. The arrow represents intraperitoneal administration of naloxone (1 mg/kg) or saline. Each point represents the mean and the vertical bars the standard errors for 10 rats.



○ β-ENDORPHIN/NALOXONE
● β-ENDORPHIN/SALINE
■ SALINE/NALOXONE
RECTAL TEMPERATURE DIFFERENTIAL (°C)
2.0
1.5
1.0
0.5
0
0.5
0
30
60
90
120
150
180
TIME (MINUTES)

Figure 14. Rectal temperature differential of intact (o) adrenalectomized (●) and hypophysectomized (■) rats receiving intracerebroventricular injections of varying doses of beta-endorphin.

Each point represents the mean value and the vertical bars the standard errors. Values in parentheses indicate the number of rats used.



○ INTACT
● ADRENALECTOMIZED
■ HYPOPHYSECTOMIZED
RECTAL TEMPERATURE DIFFERENTIAL (°C)
2.0
1.5
1.0
0.5
0
-0.5
-1.0
LOG · β-ENDORPHIN DOSE (μg)
-1.5
-1.0
0
1.0
2.0
(11)
(7)
(5)
(5)
(7)
(9)
(10)
(12)
(5)
(19)
(5)
(10)
(9)
(7)
(5)
(8)
(7)
(4)

Table 5. Corticosterone and rectal temperature differentials of intact rats 60 min post-intracerebroventricular injection of beta-endorphin.

Beta-endorphin Dose (μg)	Differential*			
	n**	Corticosterone (μg/dl)	n	Rectal temperature (° C)
0.05	5	11.8 ± 3.46 [a***]	5	0.0 ± 0.36 [a]
1.0	6	5.1 ± 3.81 [a]	6	0.3 ± 0.43 [a]
5.0	6	10.7 ± 3.03 [a]	6	0.8 ± 0.42 [a,b]
10.0	4	6.7 ± 0.94 [a]	4	1.5 ± 0.15 [b]
25.0	5	6.6 ± 1.15 [a]	6	1.3 ± 0.26 [b]
50.0	7	15.1 ± 3.95 [a]	6	1.3 ± 0.50 [b]

* Corticosterone and rectal temperature differential = 60 min post-injection value minus 0 time value, corrected for saline value and expressed as mean ± S.E.

** Number of rats.

*** Values with different superscripts (a-b) differ significantly ($P < 0.05$) within each column as determined by analysis of variance and Duncan's multiple range test.

Table 6. Thyroxine (T_4), triiodothyronine (T_3) and rectal temperature differentials of intact rats 60 min post-intracerebroventricular injection of beta-endorphin.

Beta-endorphin dose (μg)	Differential*					
	n**	T_4 (μg/dl)	n	T_3 (ng/dl)	n	Tr (° C)
1.0	6	0.05 ± 0.12 [a***]	6	-7.93 ± 1.98 [a,b]	7	0.4 ± 0.10 [a]
10.0	6	-0.32 ± 0.22 [a]	6	-10.48 ± 1.92 [a]	19	1.1 ± 0.13 [b]
25.0	6	-0.23 ± 0.20 [a]	6	-4.25 ± 1.51 [b]	7	1.2 ± 0.30 [b]

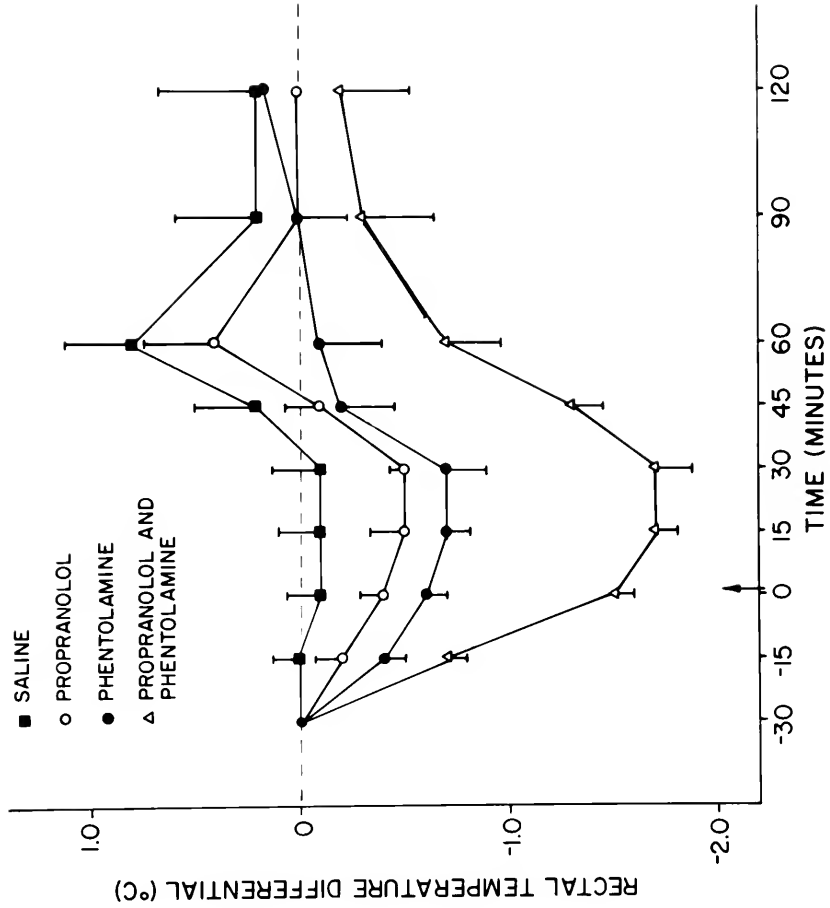
* Thyroxine, triiodothyronine and rectal temperature differential = 60 min post-injection value minus 0 time measurement, corrected for saline value and expressed as mean ± S.E.

** Number of rats.

*** Values with different superscripts (a-b) differ significantly ($P < 0.05$) within each column as determined by analysis of variance and Duncan's multiple range test.

Figure 15. Time course effects of rats pretreated with saline (■), propranolol (o), phentolamine (•) or propranolol and phentolamine (Δ) 30 min prior to intracerebroventricular administration of beta-endorphin (10 μg) at the arrow.

Each point represents the mean value and the vertical bars the standard errors for six rats.



■ SALINE
○ PROPRANOLOL
● PHENTOLAMINE
△ PROPRANOLOL AND PHENTOLAMINE
RECTAL TEMPERATURE DIFFERENTIAL (°C)
1.0
0
-1.0
-2.0
-30
-15
0
15
30
45
60
90
120
TIME (MINUTES)

resulting from simultaneous administration of propranolol and phentolamine was greater than individual injections of each drug.

Intracerebroventricular administration of B-END to rats pretreated with propranolol for 0.5 hr increased ΔTr after 30 min (Figure 15). Administration of B-END to rats pretreated with phentolamine caused the ΔTr to increase but not to the 60 min level of either the propranolol or saline groups. The ΔTr increased following IVT administration of B-END in the propranolol/phentolamine group.

Discussion

In previous reports on the effects of opioids on body temperature in rats, three major patterns appear: hyperthermia (Blasig et al., 1979), hypothermia, or a biphasic response (Clark, 1981). These different results may be explained by considering the range (0.6 μg - 0.5 mg) of B-END doses and the routes (IP, IVT, IV) of administration (Yehuda and Kastin, 1980) used in those studies. On the other hand, the doses of B-END (5 to 50 μg) used in the present study produced hyperthermic responses only when injected IVT; IV administration of the same doses did not elevate ΔTr. Although only a small percent (0.3%) of IV injected B-END crosses the blood-brain barrier (Reilly et al., 1980), the current findings strongly suggest that Tr responses induced by B-END are mediated through a central (IVT) mechanism of action.

Beta-endorphin may mediate changes in body temperature either through central thermoreceptors or through calorigenic or vasoactive hormones. This action may involve B-END alone or in combination with one or more neurotransmitters. The response obtained may depend on the dose of B-END administered. Low doses of B-END may produce opiate

effects while administration of higher doses also may induce non-opiate effects which cannot be antagonized by naloxone (Yehuda and Kastin, 1980).

No linear dose-response curve for ΔTr and B-END previously has been reported (Yehuda and Kastin, 1980). However, doses reported herein resulted in a hyperthermic response that correlated linearly with the log dose of B-END using the statistical models described above (Figure 12). This hyperthermia appears to be specific for B-END as varying doses of leucine-enkephalin and angiotensin II did not alter ΔTr.

In general, the doses (0.05-50 μg) of IVT injected B-END did not appear to change the behavior of the rats. Occasionally, high doses (25, 50 μg) of B-END resulted in quiescent rats while lower doses (5, 10 μg) excited some rats.

Antagonism of the hyperthermic effects of B-END by naloxone previously has been reported (Rudy and Yaksh, 1977; Blasig et al., 1978; Huidobro-Toro and Way, 1979) but others have found B-END-induced hyperthermia to be resistant (Cox et al., 1976) or partially resistant (Martin and Bacino, 1979) to naloxone antagonism. The data presented here (Figure 13) demonstrate the antagonistic effect of naloxone on the B-END caused hyperthermia. This suggests that naloxone-antagonized opioid receptors are involved. It is known that naloxone strongly antagonizes μ receptors but is less dominant at the other opioid (K,δ,σ) receptors (Martin, 1981). When injected alone, naloxone had little effect on body temperature. This is in agreement with Ferri et al. (1978), Holaday et al. (1978), Thornhill et al. (1978), Blasig et al. (1979), Cox et al. (1979) and Wong and Bentley (1979).

All reported that naloxone, given IP or subcutaneously, had no effect on body temperature in doses ranging from 3-40 mg/kg at room temperature.

Neither adrenalectomized or hypophysectomized rats demonstrated a dose-response relationship (Figure 14) comparable to that produced by intact rats (Figure 12) at any time interval studied. Holaday et al. (1977) found no alteration in opiate potency following IVT administration in adrenalectomized animals. Hypophysectomized rats had reduced plasma (Mueller, 1980) and brain (Kerdelhue et al., 1982) B-END-like-immunoreactivity which may alter the sensitivity of opiate receptors. Adrenal atrophy, a consequence of hypophysectomy, may alter the hormone balance necessary to produce the thermal effects of B-END.

Involvement of the hypothalamo-pituitary-adrenal axis implicates adrenal cortical hormones in mediating the Tr responses reported in this study. In addition, the findings of Herrmann (1942), Numan and Lal (1981) and Thornhill and Wilfong (1982) suggest adrenocortical involvement in opiate-induced hyperthermia. However, in the study reported herein, serum corticosterone differentials were not altered by the dose of B-END administered. Moreover, serum corticosterone differentials were not related to the Tr changes. Risch et al. (1983) reported positive correlations between plasma levels of B-END and ACTH, but not cortisol, in humans. These authors attributed this response to different adrenal receptor sensitivities to ACTH or differences in metabolic clearance rates of plasma ACTH and cortisol. These data may reflect concomitant releases of ACTH and B-END from the pituitary gland. Alternatively, B-END and ACTH may act in opposition to maintain a relatively constant body temperature (Thornhill and Wilfong, 1982).

Although serum corticosterone does not appear to be directly involved in B-END-induced hyperthermia, this hormone may have a permissive role (Gale, 1973).

Modification of endogenous levels of B-END in brain tissues by thyroid hormones has been reported by Gambert et al., 1980. The present findings suggest thyroid hormone involvement because the doses of B-END administered inhibit serum T_3 levels. In contrast, thyrotropin releasing hormone reversed B-END-induced hyperthermia in rats (Holaday et al., 1978a).

Body temperature also may be influenced by the catecholamines, epinephrine and norepinephrine (Fregly et al., 1979) which stimulate α- and β-adrenergic receptors. Blockage of these receptors with propranolol, phentolamine or both drugs in combination decreased ΔTr. Body temperature was lowered, because of tail vasodilation due to loss of vasconstrictor tone (α-receptor mediated) or decreased MR (predominantly β-receptor mediated). Nevertheless, B-END administered IVT to all of these groups increased Tr (Figure 14), suggesting that B-END-induced hyperthermia is not mediated through α or β receptors.

The data presented herein suggest a central (IVT) role for B-END-induced hyperthermia. The doses (0.05-50 μg) presented produced a linear log B-END dose-rectal temperature response relationship, specific for B-END. The actions of B-END involve μ opiate receptors. Adrenergic receptors do not appear to be involved in the hyperthermic response produced by B-END. Beta-endorphin administration had no effect on serum corticosterone; however, serum T_3 was inhibited. More detailed studies will be required to understand the interactions of these hormones.

Summary

The effects of beta-endorphin (B-END) on body temperature and the role of the adrenal and thyroid glands were examined in male, Sprague-Dawley rats in a controlled environment room at 24.5° C. Relative humidity of 50 ± 0.3% and a 12L:12D photoperiod (L = 0900 to 2100 hr) were maintained. Rectal temperature (Tr) was measured using thermistors, corticosterone and thyroid hormones by radioimmunoassay. Intracerebroventricular (IVT) administration of varying doses 0.05-50 μg of B-END resulted in a dose-related hyperthermia that began 30 min post-IVT injection and continued for an additional hr. Intravenous injections of the same doses of B-END resulted in little or no Tr response. The B-END-induced hyperthermia was antagonized by naloxone. Pretreatment with propranolol, phentolamine or both drugs in combination did not block the hyperthermia caused by B-END. Adrenalectomized and hypophysectomized rats injected IVT with B-END did not consistently increase Tr. Beta-endorphin administration had no effect on serum corticosterone; however, serum triiodothyronine was inhibited. These data suggest the hyperthermic action of B-END is mediated centrally through μ opiate receptors. Adrenergic receptors do not appear to be involved in the hyperthermic response produced by B-END.

CHAPTER 3
THE EFFECTS OF PRECONDITIONING TEMPERATURE ON PITUITARY AND BLOOD LEVELS OF BETA-ENDORPHIN IN RATS AT 32.5° C

Introduction

The data presented in Chapter 2 demonstrate that rectal temperature differentials (ΔTr) induced by intracerebroventricular (IVT) injections of beta-endorphin (B-END) were similar to those of rats preconditioned to 24.5° C and acutely (3 hr) exposed to 32.5° C (Chapter 1). The ability of B-END to alter body temperature suggests involvement in the process of physiological adaptation. Plasma levels of B-END-like-immunoreactivity (B-END-LI) increase during exposure to heat (Deeter and Mueller, 1981) and physiological stress (Millan et al., 1981; Mueller, 1981). Thornhill et al. (1980) suggest that blockade of brain and pituitary endorphins by opiate antagonists alters the rat's ability to physiologically adapt to severe changes in environmental temperature.

Using a purified preparation of corticotropin releasing factor, Vale et al. (1978) directly stimulated the *in vitro* release of B-END and adrenocorticotropic hormone (ACTH) by rat pituitary tissue. These findings suggest a common mechanism for release of B-END and ACTH from the rat pituitary gland. Co-release of ACTH and B-END from the anterior pituitary gland was observed by Guilleman et al. (1977), Mains et al. (1977), Pellefier et al. (1977) and Rossier et al. (1977). Increased glucocorticoids have been observed in rats injected with ACTH

(Evans et al., 1957) and rats exposed to environmental temperatures of 29.2° C (Attah and Besch, 1977) and 32.5° C (Gwosdow-Cohen et al., 1982, Appendix B). Like ACTH, the secretion of B-END is inhibited by glucocorticoids (Guilleman et al., 1977; Hollt et al., 1979; Wardlaw and Frantz, 1979; Wiedemann et al., 1979; Mueller, 1980).

These commonalities suggest that B-END may contribute to the rectal temperature changes observed in rats preconditioned to different environmental temperatures. Hence, the present study was conducted to evaluate the influence of preconditioning temperatures on pituitary and blood serum levels of B-END-LI in rats acutely exposed to the heat (32.5° C). Also measured were the blood serum levels of thyroid hormones and corticosterone. The results are reported here.

Materials and Methods

Animals

Sixty-four adult (2 months old), male Sprague-Dawley rats [Caw: CFE (SD)] were used in these experiments. These rats (351.3 ± 4.1 g, mean ± S.E.) were housed individually in metabolic cages (Model 4-641-000, Acme) for at least 2 weeks before experiments began. Water bottles equipped with sipper tubes were attached to each cage. Food (Laboratory Rodent Chow, Ralston Purina Company) and water were available *ad libitum*.

Environmental Room

Two environmental chambers (Model C7-88, Forma Scientific) were used in this study: one for preconditioning and one for

thermal exposures. Dry-bulb temperatures of 24.5 ± 0.1° C or 29.2 ± 0.1° C were utilized as preconditioning temperatures. Relative humidity of 50 ± 0.3% and a 12L:12D photoperiod (L = 0900 to 2100 hr) were maintained. The ambient temperature in the environmental room was monitored as described in Chapter 1.

Experimental Procedures

General. All rats were preconditioned to either 24.5° C or 29.2° C (control temperatures) for at least 2 weeks before beginning each experiment. The rats then were randomly selected and divided into equal groups for exposure to the respective control temperature or 32.5° C in standard rodent shoebox cages for 1 hr because Deeter and Mueller (1981) reported plasma levels of B-END-LI are maximal 60 min after exposure to heat. Measurements of rectal (Tr) and tail skin (Tts) temperature and body mass were made at the beginning and end of each 1 hr experimental session. Evaporative water loss and metabolic rate were measured 1 hr after temperature exposure. Immediately following these measurements, the rats were decapitated and blood was collected in plastic test tubes containing 0.5 ml of 10% ethylenediaminetetraacetic acid (EDTA) and chilled on ice. Plasma was separated by centrifugation and stored at -70° C until hormone assays were conducted. The pituitary neurointermediate lobe (NIL) was dissected from the anterior lobe (AL) and both tissues were homogenized in 0.5 ml of 1 N acetic acid. Aliquots of the homogenates were removed for protein determination (Protein Assay Kit, BioRad Laboratories) and all samples were stored at -70° C until tissue and protein assays were conducted.

Radioimmunoassays (RIA). Serum B-END-LI was measured according to the procedure of Mueller (1980). The RIA procedures used to determine serum corticosterone and thyroid hormones were described in Chapter 2.

Measurements

All measurements were performed between 0900 and 1200 hr.

Body temperature. The procedures for measurement of Tr and Tts were described in Chapter 1.

Evaporative water loss (EWL). The open-flow system used for determination of EWL in rats was detailed in Chapter 1.

Metabolic rate (MR). The oxygen consumption method for measurement of metabolic rate was described earlier (Chapter 1). Oxygen consumption was measured in one rat during each 1 hr experimental session.

Statistical Analysis

Differences between treatments were determined by analysis of variance. Duncan's multiple range test was used to compare the means. Significance was assumed when $P < 0.05$.

Results

Rats adapted to either 24.5° C or 29.2° C displayed elevated evaporative water loss (ΔEWL) and tail skin temperature (ΔTts) differentials following exposure to 32.5° C for 1 hr (Table 7); the ΔEWL and ΔTts of 24.5° C adapted rats were significantly ($P < 0.05$)

Table 7. Differential effects of metabolic rate, evaporative water loss and rectal and tail skin temperature of rats preconditioned to different environmental temperatures and exposed to 32.5° C for 1 hr.

Differential*	n**	24.5° C adapted	n	29.2° C adapted	P***
Metabolic rate ($ml/min \cdot kg^{0.75}$)	6	1.62 ± 0.37	6	1.20 ± 0.31	NS
Evaporative water loss ($mg/g \cdot hr^{-1}$)	6	3.19 ± 0.36	6	0.71 ± 0.19	< 0.01
Rectal temperature (° C)	10	0.7 ± 0.18	10	0.7 ± 0.19	NS
Tail skin temperature (° C)	10	4.6 ± 0.43	10	1.7 ± 0.43	< 0.01

* Differential = final measurement 1 hr post-exposure to 32.5° C minus initial measurement at preconditioned temperature (0 time), expressed as mean ± S.E.

** Number of rats.

*** P value denotes significant differences as determined by analysis of variance.

higher than 29.2° C adapted animals. On the other hand, the rectal temperature (ΔTr) and metabolic rate (ΔMR) differentials of both groups of rats were elevated to comparable levels following acute (1 hr) exposure to the heat (32.5° C).

The influence of preconditioning temperature on blood levels of thyroid hormones, corticosterone, and B-END-LI are shown in Table 8. The thyroid hormones, T_3 and T_4, were significantly reduced in the control group of rats adapted to 29.2° C compared to those adapted to 24.5° C. For both groups of rats acute exposure to the heat (32.5° C) did not change T_3 levels. On the other hand, 24.5° C adapted rats significantly increased T_4 levels following acute exposure to 32.5° C; T_4 levels of rats adapted to 29.2° C were not altered on exposure to 32.5° C. Serum corticosterone levels of both groups of rats were quantitatively similar at control temperatures but were significantly elevated following 1 hr in the heat (32.5° C).

Control rats adapted to 29.2° C displayed highly significant ($P < 0.01$) elevations in B-END-LI compared to 24.5° C adapted rats (Table 8). Following exposure to 32.5° C, B-END-LI of 24.5° C adapted rats was significantly elevated and was similar to that of the 29.2° C adapted control rats. Rats physiologically adapted to 29.2° C displayed a slight, but not significant, elevation in B-END-LI following acute exposure to 32.5° C.

The content of B-END-LI in the NIL of the pituitary gland of 29.2° C adapted control rats was significantly elevated compared to 24.5° C controls, but no differences were detected in the AL (Table 9). On exposure to 32.5° C, the B-END-LI of the NIL and AL was significantly elevated for both experimental groups of rats. Exposure to the

Table 8. Blood levels of thyroid hormones, corticosterone and beta-endorphin-like-immunoreactivity in rats preconditioned to different environmental temperatures and exposed to 32.5° C for 1 hr.

Hormone	24.5° C adapted		29.2° C adapted	
	Control (24.5° C)	Experimental (32.5° C)	Control (29.2° C)	Experimental (32.5° C)
Triiodothyronine (ng/dl)	52.71 ± 2.00 [a**]	56.88 ± 1.70 [a]	41.60 ± 0.96 [b]	39.17 ± 1.47 [b]
Thyroxine (μg/dl)	3.83 ± 0.12 [a]	4.40 ± 0.06 [b]	2.99 ± 0.10 [c]	3.00 ± 0.07 [c]
Corticosterone (μg/dl)	17.96 ± 0.89 [a]	23.72 ± 1.02 [b]	17.00 ± 0.67 [a]	22.03 ± 1.06 [b]
Beta-endorphin-like immunoreactivity (ng/ml)	0.24 ± 0.03 [a]	0.56 ± 0.04 [b]	0.67 ± 0.09 [b,c]	0.85 ± 0.07 [c]

* Mean ± S.E.; values are based on duplicate determinations of samples from 10 rats.

** Values with different superscripts (a–c) differ significantly ($P < 0.05$) between columns as determined by analysis of variance and Duncan's multiple range test.

Table 9. Pituitary beta-endorphin-like immunoreactivity in rats preconditioned to different environmental temperatures and acutely exposed to 32.5° C for 1 hr.

Temperature (° C)		Neurointermediate lobe*		Anterior lobe	
Precondition	Experimental	μg/gland	μg/mg protein	μg/gland	μg/mg protein
24.5	24.5	$5.47 \pm 0.27^{a**}$	32.18 ± 0.24^{d}	0.28 ± 0.01^{h}	0.22 ± 0.01^{j}
24.5	32.5	7.60 ± 0.39^{b}	40.00 ± 0.42^{e}	0.32 ± 0.01^{i}	0.27 ± 0.02^{k}
29.2	29.2	8.20 ± 0.34^{b}	51.25 ± 0.37^{f}	0.27 ± 0.01^{h}	0.19 ± 0.03^{j}
29.2	32.5	9.72 ± 0.53^{c}	54.18 ± 0.41^{g}	0.35 ± 0.01^{i}	0.29 ± 0.02^{k}

* Mean ± S.E.; values are based on duplicate determinations for 10 rats.

** Values with different superscripts (a-k) differ significantly ($P < 0.05$) within each column as determined by analysis of variance and Duncan's multiple range test.

heat resulted in elevation of the B-END-LI to similar levels in the AL and NIL for both groups of rats.

Exposure to 32.5° C for 1 hr increased ΔTr of both groups of rats by 0.7° C (Table 7). However, the B-END-LI differentials of 24.5° C and 29.2° C adapted rats were 0.32 and 0.81 ng/ml, respectively (Table 8). These ΔTr results are similar to those obtained 1 hr post-IVT injection of varying doses (5-50 μg) of B-END (Figure 11). Moreover, serum concentration of B-END for preconditioned rats (0.003-0.011 μg) was qualitatively similar to that of IVT injected rats (0.067-0.007 μg).

Discussion

Elevated MR and Tr commonly occur when animals are exposed to environmental conditions for which they are unaccustomed or nonadapted (Herrington, 1940; Yousef and Johnson, 1967) and signals the start of physiological adjustment to the new environment (Prosser, 1964). However, in the study reported here, rats adapted to 24.5° C appeared to dissipate greater amounts of heat from the tail and by evaporation of water compared to 29.2° C adapted rats. These findings agree with those of Chapter 1 which suggest that the thermoneutral zone for 24.5° C adapted rats is different from the thermoneutral zone of 29.2° C rats. The different upper critical temperatures of the thermoneutral zones of both groups of rats (as described in Chapter 1) may indicate a changed sensitivity to heat of the sensory component of the rat's thermoregulatory mechanism. This altered "heat sense" could account for the different heat dissipation responses of 24.5° C and 29.2° C adapted rats following acute exposure to 32.5° C.

The increased MR observed on exposure to heat may be the result of increased adrenal (Yousef and Johnson, 1967; Kotby et al., 1967) or thyroid (Yousef and Johnson, 1968) activity. The secretions from these glands which include thyroid hormones, catecholamines and corticosterone, all are calorigenic hormones (Evans et al., 1957; Collins and Weiner, 1968). In the present experiments (Table 8), control rats adapted to 24.5° C and 29.2° C had similar levels of serum corticosterone which indicate physiological adaptation to heat. Corticosterone increased similarly in both groups after 1 hr exposure to 32.5° C. The elevated serum corticosterone levels observed in the present study (Table 8) at 32.5° C probably contributed to the increased MR (Table 7; Kotby and Johnson, 1967; Kotby et al., 1967). Increased corticosterone secretion has been observed in rats adapted to 24.5° C and exposed to experimental temperatures of 29.2° C (Attah and Besch, 1977) and 32.5° C (Gwosdow-Cohen et al., 1982, Appendix B). It has been reported (Yousef and Johnson, 1967; Gwosdow-Cohen et al., 1982, Appendix B) that corticosterone levels peak 24 hr after exposure to heat before returning to control levels.

On physiological adaptation to heat, thyroid activity decreases (Dempsey and Astwood, 1943; Chaffee and Roberts, 1971) and MR returns to control levels (Gelineo, 1934). Yousef and Johnson (1968) attribute part of the decline in MR to the concomitant decreased thyroid activity. Similar findings have been reported by Kotby and Johnson (1967) and Kotby et al. (1967). In the present study, 29.2° C adapted rats maintained significantly reduced levels of T_3 and T_4 compared to their 24.5° C counterparts but on acute exposure to heat, the 24.5° C animals had elevated T_4 levels only. The reduced thyroid hormone

levels of 29.2° C adapted rats, compared to 24.5° C adapted rats, and the maintenance of this lowered level following exposure to 32.5° C are additional indicators of the rat's physiological adaptation to heat.

The elevated MR displayed by 29.2° C and 24.5° C adapted rats after 1 hr in the heat (Table 7) may be due to the actions of corticosterone or may represent the initial response of these rats to heat. Three hr after exposure to 32.5° C (Chapter 1), the 29.2° C adapted rats had significantly lowered MR compared to 24.5° C adapted rats. This reduction in MR in 29.2° C rats (Figure 6) may be due partially to the lower thyroid hormone levels maintained by these rats (Table 8). The resultant lower thyroid activity may be responsible for the decreased MR and heat output observed in the heat adapted animals (Cassuto and Chaffee, 1966).

Circulating levels of B-END-LI increase in the heat (Mueller, 1980; Deeter and Mueller, 1981) and in response to stressful stimuli (Mueller, 1981). Mueller (1981) suggests that the increase in B-END-LI in the blood is directly related to the intensity of the stimulus. In the experiment reported here, 29.2° C adapted rats had elevated B-END-LI compared to 24.5° C adapted animals. On exposure to heat, the levels of B-END-LI significantly increased in 24.5° C animals, but not in the 29.2° C group. Moreover, at 32.5° C both groups of rats maintained comparable levels of B-END-LI. These data suggest that 32.5° C was not a "stressful" environment for the 29.2° C adapted rats compared to the 24.5° C adapted animals.

Mueller (1980) found that approximately 95% of the rat's B-END-LI is located in the NIL. The data presented in Table 9 support these findings. The B-END-LI content of this tissue was increased in 29.2° C

adapted control rats compared to the 24.5° C controls and was elevated in both groups of rats in the heat. While Guilleman et al. (1977) suggest that changes in B-END-LI reflect changes in pituitary secretion, the data reported herein suggest that pituitary content of B-END-LI is elevated concomitantly with increases in circulating levels. This may indicate that stimuli which release B-END-LI into the bloodstream also increase the synthesis or delivery of B-END-LI to the pituitary gland (Bergland and Page, 1979).

The thyroid and corticosterone responses reported in this chapter are similar to those reported for heat adapted rats (Dempsey and Astwood, 1943; Kotby and Johnson, 1967). Preconditioning and heat exposure appear to have altered the blood and pituitary levels of B-END-LI, and these hormonal and rectal temperature responses mimic those induced by IVT injections of B-END (Chapter 2). Therefore, B-END may be responsible for the Tr increases observed in the experiments reported herein and may play a role in the process of physiological adaptation to heat.

In order to compare the response of IVT injected B-END with B-END-LI resulting from heat exposure, the metabolic clearance rate and half-life of B-END need to be estimated. Little is known about the metabolic clearance rate of B-END, but Houghten et al. (1980) reported the plasma half-life of B-END was 45 min. Skeleton (1927) estimated that a 300 g rat has 195.9 ml of water of which 13.3 ml (6.9%) is blood. Reilly et al. (1980) demonstrated that 0.3% of the B-END injected IV crosses the blood-brain barrier. From these data, it is possible to calculate the total concentration of serum B-END-LI in preconditioned and IVT injected rats. The similarity of obtained

results suggest that B-END may very likely be responsible for the rectal temperature elevations produced in these experiments.

SUMMARY OF RESULTS

1. Preconditioning temperature appears to influence the threshold or reference point for activation of thermoregulatory responses in the rat.
   a. The absolute values for rectal and tail skin temperature of 29.2° C adapted rats were maintained at a higher level compared to 24.5° C adapted rats.
   b. The thermoneutral zone of 29.2° C adapted rats is different from that of 24.5° C adapted rats.
   c. After exposure to 32.5° C for 1 hr,
      (1) Rats preconditioned to either 24.5° C or 29.2° C displayed similar metabolic rate and rectal temperature differentials.
      (2) Rats adapted to 29.2° C had lower evaporative water loss and tail skin temperature differentials compared to 24.5° C adapted rats.
   d. After exposure to 32.5° C for 3 hr,
      (1) Rats adapted to 29.2° C had lower metabolic rates, evaporative water loss and tail skin temperatures than 24.5° C adapted rats.
      (2) Rats adapted to 29.2° C increased water intake and decreased food intake compared to 24.5° C adapted rats.

(3) Rats preconditioned to either 24.5° C or 29.2° C displayed similar rectal temperature differentials.

e. The percent heat lost by evaporation at 32.5° C and 34.5° C was greater for 29.2° C adapted rats compared to 24.5° C adapted rats.

f. Compared to 24.5° C adapted rats, those rats adapted to 29.2° C displayed:

(1) Lowered serum thyroid hormones but elevated serum beta-endorphin-like-immunoreactivity (B-END-LI);

(2) Elevated B-END-LI in the neurointermediate and anterior lobes of the pituitary gland

(3) No change in serum corticosterone levels.

2. Future studies on physiological responses of rats to varying environmental temperatures should account for the rat's thermal history.

3. Intracerebroventricular (IVT) injections of beta-endorphin (B-END) between 0.05-50 μg produced a dose-related hyperthermic response manifested as increased rectal temperature; little or no response was observed from intravenous injections of the same doses.

a. Hyperthermia was evident 30 min post-IVT injection and continued for an additional hr.

b. Naloxone antagonized B-END induced hyperthermia; naloxone administered alone had no effect on rectal temperature.

c. Adrenalectomized and hypophysectomized rats administered B-END did not consistently increase rectal temperatures or result in a dose-response curve.

d. Beta-endorphin injected IVT did not change serum corticosterone levels but serum triiodothyronine was inhibited; the levels of these hormones were not related to the hyperthermia induced by B-END.

e. Beta-endorphin-induced hyperthermia was not altered by pretreatment with propranolol, phentolamine or both drugs in combination.

f. The hyperthermic action of B-END appears to be mediated centrally through μ opiate receptors; adrenergic receptors do not appear to be involved in B-END-induced hyperthermia.

4. Peptides related to B-END such as leucine-enkephalin and angiotensin II did not cause rectal temperature changes or result in dose-response relationships.

5. The rectal temperature responses induced by IVT injections of B-END in rats at 24.5° C were similar to those observed in rats exposed to 32.5° C; the concentration of B-END-LI in the bloodstream of these two groups of rats was similar.

6. A one hour exposure of rats to 32.5° C from 24.5° C increased serum corticosterone, thyroxine and B-END-LI in the blood and pituitary glands of rats.

CONCLUSIONS

The scheme of events involved in physiological adaptation (Figure 1) was modified to describe the temperature regulation system (Figure 16). In general, environmental temperature is sensed by cutaneous and deep body thermoreceptors which relay this information to the central nervous system (CNS) regulator located in the preoptic anterior hypothalamus (POAH). The POAH balances the thermal information with the predetermined or "set-point" level for regulation of body temperature and activates effector mechanisms in proportion to the deviation. These effector mechanisms (metabolic, vasomotor, sudomotor, behavioral) stimulate physiological events that return core temperature to the "control" level. Body temperature is thus regulated through a feedback loop.

Both of these schemes (Figures 1 and 16) were modified to include the data in this dissertation. The thermal environment (heat) has been the focus of the research described herein (Figure 17). Thermal information from receptors may be relayed to the POAH directly or by central nervous system (CNS) substances such as beta-endorphin (B-END). The CNS regulator stimulates effector mechanisms which are responsible for the physiological events through hormonal or neuronal stimulation. Hormones, peptides or neurotransmitters released from appropriate effector organs control intracellular events by interacting with specific receptors or enzymes of the cell. The resultant intracellular

Figure 16. Schematic of body temperature regulation.

(See text for details.)

SOURCE: Ruch and Patton (1973).

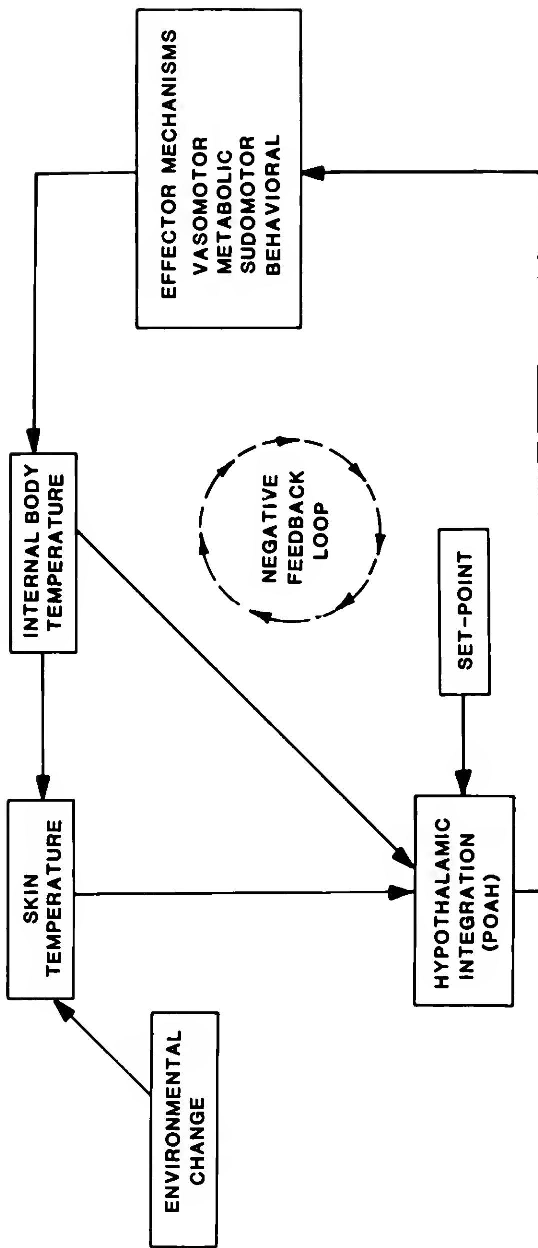
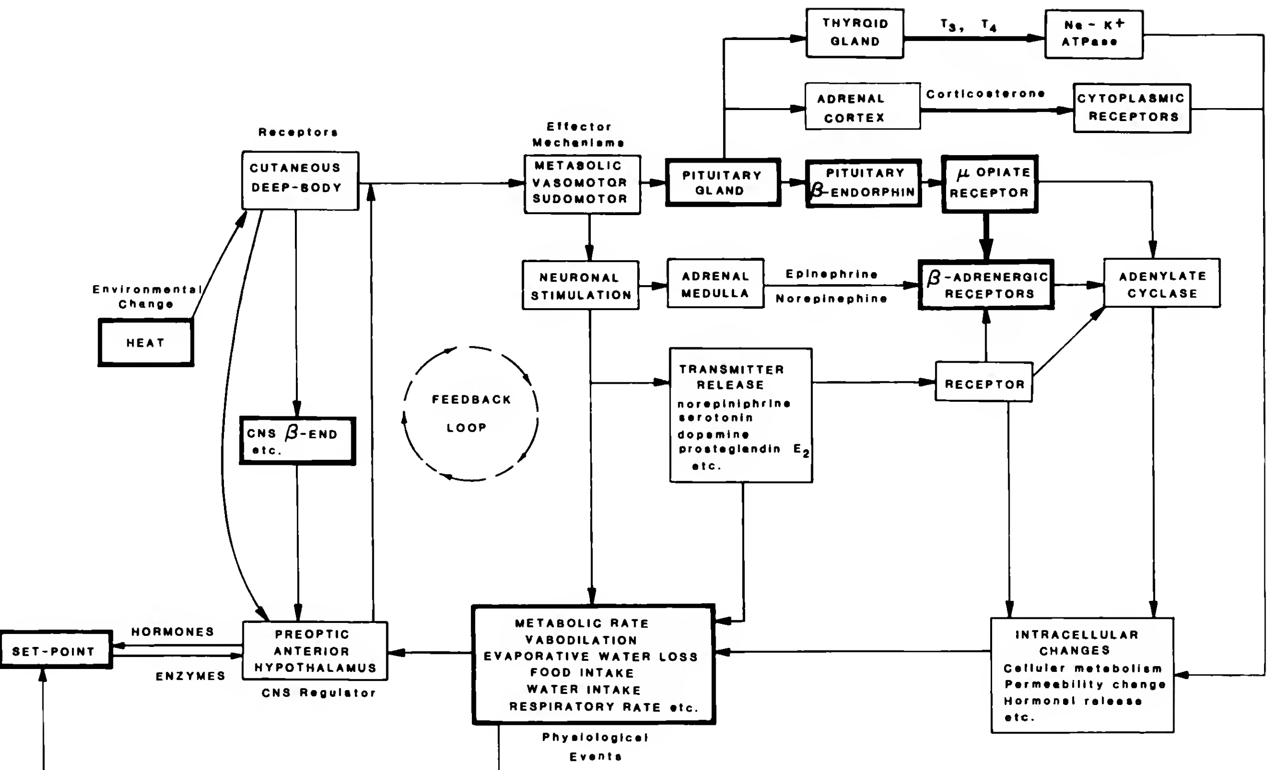

EFFECTOR MECHANISMS
VASOMOTOR
METABOLIC
SUDOMOTOR
BEHAVIORAL
INTERNAL BODY TEMPERATURE
NEGATIVE FEEDBACK LOOP
SET-POINT
SKIN TEMPERATURE
HYPOTHALAMIC INTEGRATION (POAH)
ENVIRONMENTAL CHANGE

Figure 17. Proposed diagram of the events involved in thermoregulation in the heat.

The heavily outlined boxes relate to data presented in this dissertation. (See text for details.)



Environmental Change
HEAT
Receptors
CUTANEOUS
DEEP-BODY
CNS β-END etc.
Effector Mechanisms
METABOLIC
VASOMOTOR
SUDOMOTOR
PITUITARY GLAND
PITUITARY β-ENDORPHIN
μ OPIATE RECEPTOR
THYROID GLAND
T_3, T_4
Na - K+ ATPase
ADRENAL CORTEX
Corticosterone
CYTOPLASMIC RECEPTORS
NEURONAL STIMULATION
ADRENAL MEDULLA
Epinephrine
Norepinephine
β-ADRENERGIC RECEPTORS
ADENYLATE CYCLASE
FEEDBACK LOOP
TRANSMITTER RELEASE
norepinephrine
serotonin
dopamine
prostaglandin E_2
etc.
RECEPTOR
INTRACELLULAR CHANGES
Cellular metabolism
Permeability change
Hormonal release
etc.
METABOLIC RATE
VASODILATION
EVAPORATIVE WATER LOSS
FOOD INTAKE
WATER INTAKE
RESPIRATORY RATE etc.
Physiological Events
PREOPTIC
ANTERIOR
HYPOTHALAMUS
CNS Regulator
HORMONES
ENZYMES
SET-POINT

changes produce the appropriate physiological event. The physiological events influence core temperature and the POAH through feedback mechanisms.

In the data presented herein, a higher preconditioning temperature appeared to stimulate the POAH to increase the "set-point" level for body temperature regulation in rats adapted to 29.2° C as evidenced by elevated absolute values for rectal and tail skin temperatures compared to 24.5° C adapted rats. The elevated "heat sense" of the former group may have resulted from decreased thyroid hormones (Table 8) or elevated B-END-like-immunoreactivity (B-END-LI). These hormonal changes, observed in rats adapted to 29.2° C, may have contributed to the different zone of thermal neutrality (Figure 6), upward shift in upper critical temperature (Figure 7) and changes in food and water intake patterns (Table 3) compared to 24.5° C adapted rats.

Exposure of both preconditioned groups to heat (32.5° C) for 1 hr increased metabolic rate and rectal temperature differentials similarly (Table 7), and may be due to increased corticosterone levels observed in both groups of rats (Table 8). After 3 hr, these responses plateaued at different levels (Figures 6-9) which probably reflected physiological changes due to preconditioning temperature, such as reduced thyroid hormone levels of 29.2° C adapted rats compared to rats adapted to 24.5° C (Table 8). Dissipation of heat through the tail and by evaporative cooling occurred more rapidly and differences due to preconditioning temperature were similar at one (Table 7) and three (Figures 8 and 9) hr after heat exposure. Based on these results, future studies on physiological responses of rats to heat should take into consideration the rat's thermal history.

At the thermoneutral temperature of 24.5° C, rats receiving intracerebroventricular injections (IVT) of B-END between 0.05-50 μg displayed a dose-related hyperthermic response manifested as increased rectal temperature (Figure 12); no responses were observed from intravenous injections of the same doses (Figure 11). Peptides related to B-END such as leucine-enkephalin and angiotensin II did not cause rectal temperature changes. Beta-endorphin-induced hyperthermia was antagonized by naloxone (Figure 13). Adrenalectomized and hypophysectomized rats administered B-END did not consistently increase rectal temperatures (Figure 14). Beta-endorphin injected IVT did not change serum corticosterone level (Table 5), but serum triiodothyronine was inhibited (Table 6). Pretreatment with adrenergic antagonists (propranolol, phentolamine or both drugs in combination) did not block the hyperthermia caused by B-END (Figure 15). These data suggest the hyperthermic action of B-END is mediated through μ opiate receptors; adrenergic receptors do not appear to be involved. However, more detailed studies are required to understand the interactions between hormones of the hypothalamo-pituitary axis and the adrenal and thyroid glands.

In the heat, rats preconditioned to 29.2° C displayed elevated B-END-LI in the serum (Table 8), neurointermediate and anterior lobes of the pituitary gland compared to 24.5° C adapted rats (Table 9). This suggests that activation of the peptidergic neuronal system releases B-END-LI into the bloodstream and increases the synthesis or delivery of B-END-LI to the pituitary gland. In addition, the rectal temperature differentials induced by IVT injections of B-END in rats at 24.5° C (Figure 11) were similar to those observed in rats exposed to

32.5° C (Figure 7 and Table 7) and the concentration of B-END-LI in the bloodstream of these two groups of rats was similar. The similarity of these results suggest that B-END may be involved in body temperature regulation, although the mechanism of action of B-END and resultant physiological events will require more detailed studies.

APPENDICES

APPENDIX A
TABLES OF RESEARCH DATA

Table A-1. Metabolic rate (oxygen consumption) of rats preconditioned to different environmental temperatures (24.5 or 29.2° C) and exposed to experimental temperatures between 18.0 and 34.5° C for 3 hr.

Experimental temperature (° C)	Metabolic rate* (ml O_2 consumed/min · $kg^{0.75}$)				P****
	n**	24.5° C adapted group	n	29.2° C adapted group	
18.0	10	$20.85 \pm 0.26^{a,d***}$	16	17.47 ± 0.32^{e}	< 0.05
20.0	18	17.86 ± 0.32^{b}	24	15.90 ± 0.23^{f}	< 0.05
22.2	10	14.65 ± 0.68^{c}	14	15.32 ± 0.41^{f}	NS
24.5	20	15.03 ± 0.37^{c}	28	14.52 ± 0.39^{f}	NS
27.0	10	14.07 ± 0.47^{c}	18	14.68 ± 0.42^{f}	NS
29.2	20	18.22 ± 0.53^{b}	28	15.19 ± 0.31^{f}	< 0.05
32.5	20	19.82 ± 0.42^{d}	28	18.88 ± 0.44^{g}	NS
34.5	10	21.48 ± 0.58^{a}	18	19.59 ± 0.64^{g}	< 0.05

* Values expressed as mean ± S.E.

** Number of rats.

*** Values with different superscripts (a-g) differ significantly ($P < 0.05$) within each column as determined by analysis of variance and Duncan's multiple range test.

**** P values denote significant differences between groups as determined by analysis of variance.

Table A-2. Rectal temperature differential of rats preconditioned to different environmental temperatures (24.5 or 29.2° C) and exposed to experimental temperatures between 18.0 and 34.5° C for 3 hr.

Experimental temperature (° C)	Rectal temperature differential* (° C)				P****
	n**	24.5° C adapted group	n	29.2° C adapted group	
18.0	10	$-1.0 \pm 0.18^{a***}$	16	$-0.7 \pm 0.13^{g,h}$	NS
20.0	18	-0.4 ± 0.12^{b}	24	-1.1 ± 0.09^{g}	< 0.05
22.2	10	$-0.1 \pm 0.22^{b,c}$	14	$-0.9 \pm 0.18^{g,h}$	< 0.05
24.5	20	-0.4 ± 0.11^{b}	28	$-0.6 \pm 0.11^{g,h}$	NS
27.0	10	0.2 ± 0.14^{c}	18	-0.5 ± 0.18^{h}	< 0.05
29.2	20	0.8 ± 0.20^{d}	28	0.1 ± 0.15^{i}	< 0.05
32.5	20	1.4 ± 0.21^{e}	28	1.6 ± 0.16^{j}	NS
34.5	10	2.7 ± 0.27^{f}	18	2.1 ± 0.28^{j}	NS

* Rectal temperature differential = final rectal temperature minus initial rectal temperature and expressed as mean ± S.E.

** Number of rats.

*** Values with different superscripts (a-j) differ significantly ($P < 0.05$) within each column as determined by analysis of variance and Duncan's multiple range test.

**** P values denote significant differences between columns as determined by analysis of variance.

Table A-3. Tail skin temperature differential of rats preconditioned to different environmental temperatures (24.5 or 29.2° C) and exposed to experimental temperatures between 18.0 and 32.5° C for 3 hr.

Experimental temperature (° C)	Tail skin temperature differential* (° C)				P****
	n**	24.5° C adapted group	n	29.2° C adapted group	
18.0	10	-6.2 ± 0.51[a***]	10	-9.0 ± 0.45[e]	< 0.05
20.0	10	-5.1 ± 0.35[a]	10	-8.1 ± 0.19[e]	< 0.05
24.5	10	-1.3 ± 0.37[b]	10	-4.9 ± 0.21[f]	< 0.05
29.2	10	2.7 ± 0.37[c]	10	0.0 ± 0.15[g]	< 0.05
32.5	10	4.4 ± 0.45[d]	10	1.6 ± 0.16[h]	< 0.05

* Tail skin temperature differential = final tail skin temperature minus initial tail skin temperature and expressed as mean ± S.E.

** Number of rats.

*** Values with different superscripts (a-h) differ significantly ($P < 0.05$) within each column as determined by analysis of variance and Duncan's multiple range test.

**** P values denote significant differences between groups as determined by analysis of variance.

Table A-4. Evaporative water loss differential of rats preconditioned to different environmental temperatures (24.5 or 29.2° C) and exposed to experimental temperatures between 18.0 and 34.5° C for 1 hr.

Experimental temperature (° C)	Evaporative water loss differential* (mg/g · hr^{-1})				P****
	n**	24.5° C adapted group	n	29.2° C adapted group	
18.0	10	0.24 ± 0.20[a***]	16	-0.57 ± 0.24[d]	< 0.01
20.0	18	0.51 ± 0.20[a]	24	-0.77 ± 0.20[d]	< 0.01
22.2	10	0.28 ± 0.26[a]	14	-0.68 ± 0.25[d]	< 0.01
24.5	20	0.00 ± 0.09	28	-0.87 ± 0.21[d]	--
27.0	10	0.81 ± 0.28[a,b]	18	-0.59 ± 0.33[d]	< 0.01
29.2	20	1.18 ± 0.16[b]	28	0.00 ± 0.15	--
32.5	20	3.19 ± 0.36[c]	28	0.71 ± 0.19[e]	< 0.01
34.5	10	4.11 ± 0.86[c]	18	2.91 ± 0.42[f]	< 0.01

* Evaporative water loss differential = experimental evaporative water loss minus preconditioned evaporative water loss and expressed as mean ± S.E.

** Number of rats.

*** Values with different superscripts (a-f) differ significantly ($P < 0.05$) within each column as determined by analysis of variance and Duncan's multiple range test.

**** P values denote significant differences between groups as determined by analysis of variance.

Table A-5. Comparison of intravenous (IV) and intracerebroventricular (IVT) injections of varying doses of beta-endorphin on body temperature changes 60 min post-injection.

Beta-endorphin Dose (μg)	Rectal temperature differential* (° C)			
	n**	Intravenous injections	n	Intracerebroventricular injections
0.05	6	-0.5 ± 0.23	7	-0.1 ± 0.13
1.0	6	0.2 ± 0.32	7	0.4 ± 0.24
5.0	6	-0.1 ± 0.12	12	0.9 ± 0.17
10.0	6	0.1 ± 0.30	19	1.2 ± 0.15
25.0	6	-0.2 ± 0.26	7	1.1 ± 0.13
50.0	6	0.3 ± 0.19	8	1.2 ± 0.11

* Rectal temperature differential = rectal temperature 60 min post-beta-endorphin injection minus rectal temperature at 0 time, corrected for saline values and expressed as mean ± S.E.

** Number of rats.

Table A-6. Time course effects on rectal temperature of beta-endorphin followed by saline, beta-endorphin followed by naloxone and saline followed by naloxone in rats at 24.5° C. Beta-endorphin (10 µg) or saline (5 µl) were injected intracerebroventricularly (IVT). Naloxone (1 mg/kg) or saline (0.3 ml) were injected intraperitoneally at the arrow.

Time post-IVT injection (min)	Beta-endorphin / naloxone	Beta-endorphin / saline	Saline / naloxone
30	0.8 ± 0.26 [a,A**]	1.1 ± 0.26 [c,A]	0.1 ± 0.17 [e,B]
60	1.4 ± 0.35 [a,A]	1.2 ± 0.27 [c,A]	0.0 ± 0.15 [e,B]
75	0.1 ± 0.30 [b,A]	1.1 ± 0.23 [c,B]	0.0 ± 0.25 [e,A]
90	-0.3 ± 0.30 [b,A]	0.7 ± 0.23 [c,B]	-0.1 ± 0.28 [e,A]
120	0.1 ± 0.29 [b,A]	0.7 ± 0.17 [c,B]	0.2 ± 0.39 [e,A,B]
150	0.1 ± 0.37 [b,A]	0.5 ± 0.18 [d,A]	0.4 ± 0.39 [e,A]
180	0.3 ± 0.33 [b,A]	0.9 ± 0.25 [c,A]	0.7 ± 0.37 [f,A]

* Rectal temperature differential = rectal temperature 60 min post-injection minus rectal temperature at 0 time, corrected for saline values and expressed as mean ± S.E. for 10 rats.

** Values with different superscripts differ significantly ($P < 0.05$) within (a-f) each column and between (A-B) columns, as determined by analysis of variance and Duncan's multiple range test.

Table A-7. Rectal temperature differentials* obtained from intact, adrenalectomized and hypophysectomized rats receiving post-intracerebroventricular injections of varying doses of beta-endorphin.

Beta-endorphin dose (μg)	n**	Intact	n	Adrenalectomized	n	Hypophysectomized
0.05	7	$-0.1 \pm 0.13^{a,A***}$	5	$-0.1 \pm 0.26^{c,A}$	11	$0.1 \pm 0.18^{e,A}$
1.0	7	$0.4 \pm 0.24^{a,b,A}$	5	$0.5 \pm 0.15^{c,d,A}$	9	$0.3 \pm 0.19^{e,f,A}$
5.0	12	$0.9 \pm 0.17^{b,A}$	5	$0.7 \pm 0.33^{c,d,A,B}$	10	$0.3 \pm 0.24^{e,f,B}$
10.0	19	$1.2 \pm 0.15^{b,A}$	5	$0.4 \pm 0.36^{c,d,B}$	10	$0.1 \pm 0.14^{e,B}$
25.0	7	$1.1 \pm 0.13^{b,A}$	5	$0.1 \pm 0.38^{c,d,B}$	9	$0.6 \pm 0.35^{f,A}$
50.0	8	$1.2 \pm 0.11^{b,A}$	4	$-0.2 \pm 0.38^{c,B}$	7	$0.2 \pm 0.10^{e,f,B}$

* Rectal temperature differential = rectal temperature 60 min post-injection minus rectal temperature at 0 time, corrected for saline value and expressed as mean ± S.E.

** Number of rats.

*** Values with different superscripts differ significantly ($P < 0.05$) within (a-f) each column and between (A-B) columns as determined by analysis of variance and Duncan's multiple range test.

Table A-8. Rectal temperature differential of rats administered saline, propranolol (6 mg/kg), phentolamine (6 mg/kg) or propranolol and phentolamine 30 min prior to intracerebroventricular injections of beta-endorphin.

Time post-beta-endorphin injection (min)	Rectal temperature differential* (° C)			
	Saline	Propranolol	Phentolamine	Propranolol and phentolamine
-15	0.0 ± 0.13 [a]**	-0.2 ± 0.13 [a,b]	-0.4 ± 0.09 [b,c]	-0.7 ± 0.07 [c]
0	-0.1 ± 0.17 [a]	-0.4 ± 0.11 [a,b]	-0.6 ± 0.09 [b]	-1.5 ± 0.08 [c]
15	-0.1 ± 0.20 [a]	-0.5 ± 0.17 [a,b]	-0.7 ± 0.13 [b]	-1.7 ± 0.10 [c]
30	-0.1 ± 0.23 [a]	-0.5 ± 0.05 [a]	-0.7 ± 0.20 [b]	-1.7 ± 0.17 [b]
45	0.2 ± 0.31 [a]	-0.1 ± 0.16 [a]	-0.2 ± 0.23 [a]	-1.3 ± 0.14 [b]
60	0.8 ± 0.32 [a]	0.4 ± 0.35 [a]	0.0 ± 0.30 [b]	-0.7 ± 0.26 [b]
90	0.2 ± 0.38 [a]	0.0 ± 0.30 [a]	0.2 ± 0.38 [a]	-0.3 ± 0.35 [a]
120	0.2 ± 0.46 [a]	0.0 ± 0.28 [a]	0.3 ± 0.40 [a]	-0.2 ± 0.31 [a]

* Rectal temperature differential = rectal temperature at each time minus rectal temperature 0 time, corrected for saline value and expressed as mean ± S.E. for six rats.

** Values with different superscripts (a-c) differ significantly ($P < 0.05$) between columns as determined by analysis of variance and Duncan's multiple range test.

APPENDIX B
RADIOIMMUNOASSAY (RIA) OF SERUM
CORTICOSTERONE IN RATS

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Introduction

Animals exposed to conditions for which they are unaccustomed or nonadapted exhibit responses similar to those resulting from ACTH stimulation (Collins and Weiner, 1968) including increased levels of glucocorticoids and mineralocorticoids. Corticosteroids, in particular, have been shown to be sensitive and reliable indicators of an animal's internal environment (Hennessy et al., 1979). Thus, it is common practice (Kotby and Johnson, 1967; Attah and Besch, 1977) to use fluctuations in corticosteroid levels as indicators of physiological stress.

Fluorometric (Mattingly, 1962; Glick et al., 1964) or competitive protein-binding (Murphy, 1967) techniques do not accurately measure levels of corticosterone in serum. Moreover, the development of a radioimmunoassay to measure corticosterone levels in laboratory animals has been difficult because of antibody cross-reaction (Gomez-Sanchez et al., 1975; Altland and Rattner, 1979), large sample requirements, and the need for chromatographic purification of samples. Recent availability of an antiserum containing a high affinity for and specificity to corticosterone has enabled the development of a reliable radioimmunoassay for measuring corticosterone in rat serum. This assay and its validation are described here.

Materials and Methods

Animals. Male and female Sprague-Dawley rats [Caw: CFE (SD)] were housed in metabolic cages (Acme, Model 4-641-000) and floor space was equal to the recommended value (Committee on the Care and Use of

Laboratory Animals, 1978). Food (Purina Laboratory Chow) and water were available *ad libitum*.

Surgical procedures. Five female rats were ovariectomized (O); five females were adrenalectomized-ovariectomized (A/O); and six males were adrenalectomized (A). All surgical procedures were performed through the bilateral dorsal approach using ether anesthesia. Following adrenalectomy, animals were maintained on a 0.9% NaCl drinking solution, available *ad libitum*. Completeness of adrenalectomy was verified at necropsy and by serum corticosterone levels (Krieger et al., 1979). A group of five intact females (If) and a group of seven intact males (Im) were used as controls. Rats were decapitated between 0900 and 1100 hr; blood was collected in culture tubes and serum was separated and frozen.

Experimental conditions. Radioimmunoassay of serum or plasma corticosterone was performed on rats from two different experimental conditions:

1. *A, O, A/O, and I groups*. Control temperature of 25.0 ± 1.0°, 50 ± 5% relative humidity, and a 12L:12D photoperiod (L = 0600 to 1800 hr) were maintained until decapitation, 10 days after surgery for all animals except those in the A group. The A animals were individually caged in a controlled environment room (Forma Scientific, Model C7-88) for 4 weeks following surgery. The O, A/O, and I animals were caged in pairs.

2. *Heat-stressed group*. The 12 male rats used in this experiment were caged individually in a controlled environmental room. Relative humidity of 50 ± 2% and a 12L:12D photoperiod (L = 0900 to

2100 hr) were maintained for all experiments. Continuous monitoring of the ambient temperature in the environmental room was accomplished through thermistors (YSI, Model 43TD) interfaced with a data acquisition system (Esterline Angus, Model PD2064). The dry-bulb temperature was maintained at either 24.5° (control) or 32.5° (experimental). The animals were at 24.5° for 10 days followed by acute (1-2 days) exposure to heat (32.5°). Blood samples were collected by heart puncture between 0900 and 1100 hr into heparinized tubes within 40 sec of opening the animal's cage. It previously has been reported (Zimmerman and Critchlow, 1967) that plasma corticosterone is not elevated within 4 min of cage opening. The blood was centrifuged and the plasma was frozen.

Reagents. Radioactive corticosterone [1,2,6,7-^{3}H(N)] with a specific activity of 82.1 Ci/mmol (New England Nuclear, NET-399) and corticosterone standard (Sigma Chemical) were used. Charcoal (Sigma Chemical), dextran T-70 (Pharmacia), gelatin (Sigma Chemical), and ethanol (U.S. Industrial Chemical) are required reagents. Sephadex LH-20 (Sigma Chemical), benzene (Baker Chemical), and methanol (Burdick and Jackson) were used to compare alcohol extraction procedures.

Solutions. Phosphate-buffered saline with gelatin (PBS-g), 1.0 M, pH 7.2, containing 0.1% gelatin and 0.85% NaCl, was used in the assay procedure. The charcoal suspension, for separating free from bound steroid, was composed of 0.625 g charcoal and 0.0625 g dextran in 100 ml of PBS-g buffer. The scintillation fluid consisted of a 2:1 aqueous counting scintillant (ACS, Amersham):toluene (Mallinckrodt) solution. The anticorticosterone-BSA-3 antiserum 337 was produced

in rabbits. The affinity constant for this antiserum was calculated from a Scatchard plot (Al-Dujaili et al., 1981).

Assay procedure. A previously reported assay procedure (Chen et al., 1978) was modified as follows: the serum was extracted with 200 µl of ethanol from a freshly opened bottle. For rats, 10 µl of serum was sufficient. The assay tubes containing serum were agitated with a vortex mixer and allowed to stay at room temperature for 10 min. Serum proteins were pelleted by centrifugation (Beckman, Model TJ-6) at 2500 rpm for 10 min; 50 µl of the supernatant was used for assay.

The solvent was evaporated by passing a stream of dry air over the samples, which were heated in a water bath at 60°. The samples were resuspended in 350 µl of PBS-g buffer, vortexed gently, and allowed to stand at room temperature for 20 min. One hundred microliters of a 1:2000 dilution of antiserum was added to each tube and vortexed gently. The antiserum dilution of 1:2000 was sufficient to bind 40% of the [^{3}H] corticosterone added. Twenty min later, 0.05 ml of radioactive corticosterone (10,000 cpm) in PBS-g buffer was added and each tube vortexed.

After incubation overnight at 4°, the tubes were placed in a cold room (4°) and 200 µl of charcoal suspension was added while stirring. After 10 min of incubation, the tubes were centrifuged at 2500 rpm for 10 min. Using a dilutor (Labindustries, Model 81001), 300 µl of supernatant was transferred to a scintillation counting vial with 3 ml of ACS-toluene scintillation fluid and vortexed. The scintillation vials were allowed to settle in a cool dark place (4°) for 15 min prior to counting (Packard, Model 3320). All vials were counted for 1 min.

Pooled plasma and serum samples from rats in each of four groups (A, Im, heat stressed, and control) were assayed after ethanol extraction alone, methanol extraction alone, and after column chromatography purfication. Methanol extraction involved the same procedure described above for ethanol extraction. After ethanol extraction, the dried samples were subjected to Sephadex LH-20 column chromatography using benzene:methanol (85:15) as eluant (Pattison et al., 1974). After collection, fraction samples (250 μl) were dried and residues were analyzed for corticosterone by radioimmunoassay.

Standard curve. A standard curve was prepared by assaying varying volumes of corticostèrone standard (10 pg/μl) between 10 and 1000 pg. The standard curve (Figure B-1) was plotted according to the logit transformation (Rodbard, 1974). Calculations were made with a programmable calculator (Texas Instruments, Model TI-59) interfaced with a printer (Texas Instruments, PC-100A). Results were expressed as micrograms corticosterone/100 ml serum or plasma.

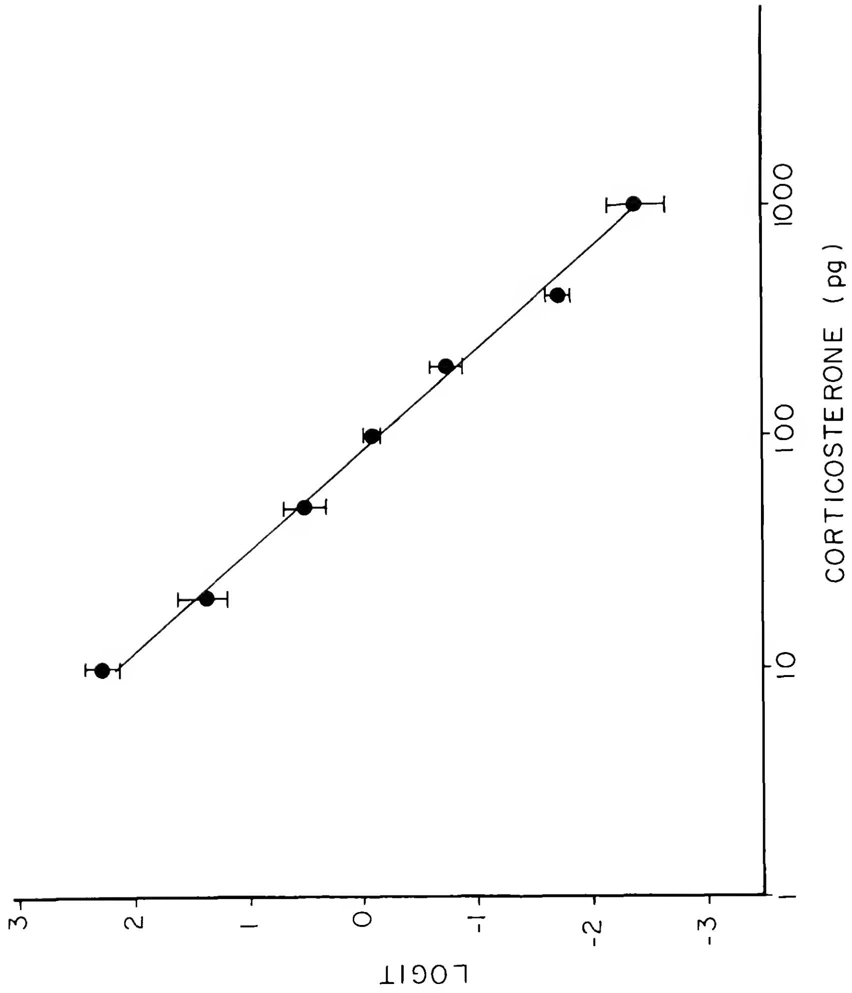
Recovery error. Using the same extraction procedure, corticosterone was recovered from a representative subset of samples equalling about 10% of the total number of unknown samples.

Various amounts of corticosterone (0.5 to 50 ng) were added to 200 μl of serum from each of six group A rats. The samples were vortexed, extracted, and assayed for corticosterone levels.

Cross-reactions. The percentage cross-reactivity was calculated as C/X x 100 where C is the amount of corticosterone and X is the

Figure B-1. Standard curve for corticosterone.

Each point represents the mean value and the vertical bars the standard errors.


LOGIT
3
2
1
0
-1
-2
-3
10
100
1000
CORTICOSTERONE (pg)

amount of cross-reacting steroid at which binding of labeled corticosterone is reduced by 50% (Al-Dujaili et al., 1981).

Statistical analysis. Comparisons between groups were determined by analysis of variance. The paired comparison t test was utilized to confirm parallelism of the slopes of the dilution curves of the A and Im groups with the standard curve for corticosterone. Significance was assumed when $P < 0.05$.

Results

Antiserum specificity. Data on the cross-reaction of antiserum with corticosterone and other steroids are presented in Table B-1. The antiserum proved to be highly specific for corticosterone. The affinity constant for this antiserum was 1.91×10^{10} liters/mole.

Recovery of added corticosterone. Recoveries averaged 69.96 ± 0.42% (mean ± SE) and all samples were corrected for this error. The relationship between the amount of corticosterone assayed compared to the amount added to the A sera is described in Figure B-2.

Precision. Sera unknowns from A and Im rats, assayed in serial dilution (10, 20, 40, 60, 80 µl), paralleled the standard curve (Table B-2). Intrassay variability expressed as a coefficient of variation was 3.67 ± 0.75% at 50 pg of corticosterone. Interassay variability with a mean corticosterone value of 51.15 pg was 6.98 ± 1.01%. Duplicates of 17 assays were used in these calculations (Rodbard, 1974).

Table B-1. The cross-reaction of anti-corticosterone-BSA-3 antiserum to corticosterone and a variety of steroids.

Compound	Percentage cross-reaction
Corticosterone	100.000
Deoxycorticosterone	7.080
11-β-Hydroxyprogesterone	7.000
Progesterone	0.952
11-α-Hydroxyprogesterone	0.321
11-Deoxycortisol	0.200
21-Deoxycortisol	0.160
Cortisol	0.114
20-β-Hydroxyprogesterone	0.053
Cortisone	0.033
Testosterone	0.007
20-α-Hydroxyprogesterone	0.006
17-Hydroxyprogesterone	0.005
Dehydroepiandrosterone	0.004
17-β-Estradiol	0.003
Aldosterone	0.002
Estriol	0.001
Estrone	< 0.001
Cholesterol	< 0.001

Figure B-2. Values for recovered amounts of corticosterone added to adrenalectomized rat sera.

Each point represents the mean value and the vertical bars tne standard errors.

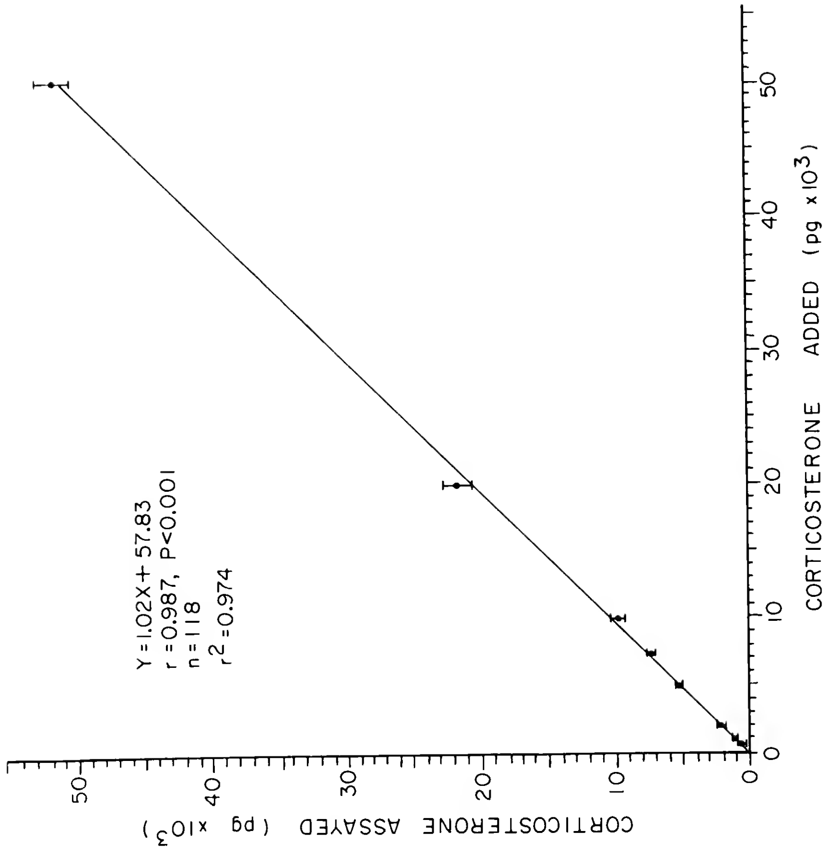

Y = 1.02X + 57.83
r = 0.987, P<0.001
n = 118
r2 = 0.974
CORTICOSTERONE ADDED (pg x10^3)
CORTICOSTERONE ASSAYED (pg x10^3)
0
10
20
30
40
50

Table B-2. Characteristics of regression equations obtained from serum samples for adrenalectomized (A) and intact male (Im) rats assayed in serial dilution*

Group	Serum corticosterone (μg/100 ml)	Slope (m)	Correlation coefficient (r)	Coefficient of determination (r^2)
Corticosterone standard	--	-2.37	-0.998	0.996
Im	19.08 ± 0.68	-2.46	-0.967	0.935
A	0.64 ± 0.04	-2.26	-0.988	0.976

* Serial dilutions: 10, 20, 40, 60, 80 μl. For each dilution, triplicate serum samples were assayed for each rat in the group. There were no significant differences between any of the compared slopes.

Sensitivity. This is defined as the smallest amount of corticosterone that is significantly different from that in the zero standard. The sensitivity of this assay was 9.48 ± 0.60 pg/assay tube.

Physiological responses. Serum obtained from the A group (0.64 ± 0.04 μg/100 ml; n = 33) and the A/O group (3.59 ± 0.40; n = 40) of rats yielded significantly ($P < 0.001$) lower corticosterone values than either If (15.26 ± 2.58; n = 15), Im (19.08 ± 0.68; n = 47), or O (24.12 ± 4.43; n = 22) animals. The If values are significantly lower than both Im ($P < 0.001$) and O ($P < 0.05$) groups; and the Im values are significantly lower than the O ($P < 0.01$) group. Corticosterone concentrations in A sera was significantly ($P < 0.001$) decreased below that of the A/O sera.

The heat-stressed (32.5°) rats (48.48 ± 4.21 g/100 ml; n = 14) displayed significantly ($P < 0.001$) elevated plasma corticosterone levels compared to control rats at 24.5° (21.31 ± 2.02; n = 24). These control plasma values are not significantly different from those obtained from I and O sera but are increased ($P < 0.05$) over the If values.

There were no significant differences between corticosterone values obtained from ethanol extraction alone compared to methanol extraction alone and column chromatography purification (Table B-3).

Discussion

In many of the referenced experiments (Fortier, 1958; Mattingly, 1962; Critchlow et al., 1963; Glick et al., 1964; Scheving and Pauly, 1966; Murphy, 1967; Zimmerman and Critchlow, 1967; Dallman et al.,

Table B-3. Comparison of corticosterone values obtained from ethanol extraction alone, methanol extraction alone, and from chromatographic purification.

Group	Ethanol extraction (μg/100 ml)	Methanol extraction (μg/100 ml)	Chromatographic purification (μg/100 ml)
Intact males (Im)	19.04 ± 1.24*	19.16 ± 1.19	18.98 ± 0.80
Adrenalectomized (A)	0.84 ± 0.14	0.80 ± 0.09	0.87 ± 0.08
Control (24.5°)	20.63 ± 2.20	21.29 ± 2.68	21.13 ± 1.42
Heat stressed (32.5°)	39.02 ± 1.72	38.07 ± 2.22	40.34 ± 2.44

* Mean ± SE; values are based on triplicate determinations for each sample.

1972; Attah and Besch, 1977; Hennessy et al., 1979) corticosterone was measured utilizing a fluorometric technique. The wide range of reported control values for male rats, from about 13 μg/100 ml (Fortier, 1958) to 25 μg/100 ml (Scheving and Pauly, 1966), is probably due to this technique. The radioimmunoassay reported here for control male rats is less variable.

Serum corticosterone levels of I, O, A/O, and A rats are similar to those previously reported (Fortier, 1958; Zimmerman and Critchlow, 1967; Dallman et al., 1972; Attah and Besch, 1977; Krieger et al., 1979). The specificity of corticosterone antiserum was very high and interference from other steroids was variable (Table B-1). Because no significant differences in corticosterone levels were observed between chromatographic purification and ethanol or methanol extraction (Table B-3), the observed cross-reactions for deoxycorticosterone (7.08%) and 11-β-hydroxyprogesterone (7.00%) do not appear to affect the usefulness of this RIA as a sensitive and precise technique for studying either serum or plasma corticosterone.

The large affinity of corticosterone antiserum for corticosterone allowed for greater sensitivity (10 pg) and made small sample (10-100 μl) use possible. Because chromatographic purification of the extract was unnecessary (Table B-3), the assay was easily performed. The intra- and interassay variability and recovery level of 70% contribute to the high reproducibility of this assay. As this RIA also is more specific than the competitive protein-binding (Murphy, 1967) fluorometric (Mattingly, 1962; Glick et al., 1964), and other RIA techniques (Gomez-Sanchez et al., 1975; Altland and Rattner, 1979), it should be the preferred method for measuring serum or

plasma corticosterone. The ease of this technique allows handling of large numbers of samples; one person can assay 400 samples per week.

It has been reported that plasma-free corticosteriods reach 0% of control levels in 4 hr (Fortier, 1958) or that they are maximally reduced in 4-6 hr (Dallman et al., 1972) postadrenalectomy in rats. It also has been shown (Krieger et al., 1979) that there are no differences in corticosterone levels between plasma samples taken at 12 hr and 28 days following adrenalectomy. In the present study, ovariectomy and adrenalectomy were performed 10 days prior to decapitation in the O and A/O groups.

Plasma corticosterone levels increased significantly ($P < 0.001$) on exposure to heat. Similar observations have been reported (Attah and Besch, 1977) in rats exposed to temperatures of 29.2 and 34.0°. Increased adrenal activity is associated with the onset of stress and results from the stress-stimulated release of ACTH, which helps the animal to adjust physiologically (Collins and Weiner, 1968). The experimental temperature (32.5°) used in this experiment is, therefore, considered stressful to these animals. The results further support the applicability of this radioimmunoassay for measuring corticosterone in physiological studies using rats.

The control values in the heat experiment are similar to some previously reported (Attah and Besch, 1977) but are higher than others (Fortier, 1958). This variation may be accounted for by the technique used for corticosterone measurement or alterations in biorhythms due to time of blood sampling. Influences of daily biorhythms do not appear to be responsible for this variation (Scheving and Pauly, 1966).

Also, in the present study, the effect of biorhythms was minimized by collecting blood samples at the same time of day from all groups.

Corticosterone levels of male rats (intact 19.08 ± 0.68 μg/100 ml; control 21.31 ± 2.02) were significantly ($P < 0.001$) elevated above the values of the females (15.26 ± 2.58). These results indicate the existence of sex differences in peripheral corticosterone levels of the rat as previously reported (Critchlow et al., 1963). Other reports (Colby and Kitay, 1972; Ogle and Kitay, 1977) suggest that changes in serum corticosterone are associated with reproductive function and status of the rat. The gonadal hormones mediate corticosterone secretion at several levels, including the hypothalamus, pituitary, liver, and the adrenal cortex (Critchlow et al., 1963; Colby and Kitay, 1972). The elevated corticosterone levels (24.12 ± 4.43) found in ovariectomized rats may result from altered adrenal metabolism (Shaikh and Shaikh, 1975).

Summary

A radioimmunoassay for corticosterone was developed and characterized using corticosterone antiserum (377, Niswender) and a simple ethanol extraction procedure. The antiserum appeared to be highly specific for corticosterone. Intraassay variability was 3.67 ± 0.75% (mean ± SE) at 50 pg of corticosterone; interassay variability with a mean value of 51.15 pg was 6.98 ± 1.01%. Assay sensitivity was 9.48 ± 0.60 pg. Utilizing this assay, serum obtained from adrenalectomized and adrenalectomized-ovariectomized rats yielded lower corticosterone values than serum from intact or ovariectomized rats. Intact females had lower corticosterone values than intact males. Rats exposed to

elevated temperature (32.5°) displayed significantly ($P < 0.001$) elevated plasma corticosterone levels (48.48 ± 4.37 μg/100 ml) compared to control (24.5°) animals (21.31 ± 2.02 μg/100 ml). The high specificity, sensitivity, precision, recovery level, and ease of this technique make it useful for the study of either serum or plasma corticosterone.

APPENDIX C
THE USE OF CEREBRAL VENTRICULOGRAPHY FOR VERIFICATION OF INTRACEREBROVENTRICULAR (IVT) CANNULA PLACEMENT IN THE LIVE RAT

Introduction

Intracerebroventricular (IVT) cannulae provide a useful means of administering substances to experimental animals. However, development of cerebral ventriculography in the live rat has been difficult because of radioactivity and lethality of radiopaque contrast media and the non-availability of an x-ray unit capable of radiographing small objects (Peterson, 1975). Heyes et al. (1983) described a technique using the radioactive contrast media, thorium, but radioactive substances pose difficulties in handling and may interfere with experimental results.

Necropsy commonly has been used to verify IVT cannula placement but, because of recent improvements in these areas, a technique has been developed for verification of IVT cannula placement in the live rat. Verifying correct cannula placement in rats prior to an experiment could be time and cost efficient and would provide the basis for teaching this surgical procedure to others. The technique described here does not cause problems of earlier methods but provides the benefits of cerebral ventriculography.

Materials and Methods

Animals. Eight adult (5 months old), male Sprague-Dawley rats [Caw: CFE (SD)] were used in these experiments. The rats (515.0 ± 7.4 g, mean ± S.E.) were housed individually in metabolic cages (Model 4-641-000, Acme) in a controlled environment room at the dry-bulb temperature of 24.5 ± 0.1° C, relative humidity of 50 ± 0.3% and 12L:12D photoperiod (L = 0900 to 2100 hr) for at least 2 weeks before beginning experiments. Water bottles equipped with sipper tubes

were attached to each cage. Food (Laboratory Rodent Chow, Ralston Purina Company) and water were available *ad libitum*.

Intracerebroventricular Cannulae. Intracerebroventricular injections were through stainless steel cannulae implanted into the right lateral ventricle while each rat was under ether anesthesia at least 72 hr before experiments began. Cannula placement was determined by the de Groot system as modified by Pellegrino et al. (1979). Correct positioning of each cannula was verified initially by the backward flow of cerebrospinal fluid. Further verification was obtained at necropsy.

Drugs. Ketamine (ketaset hydrochloride, Bristol Laboratories) and xyalazine (rompum, Haver-Lockhart Laboratories) were used as anesthetics. Diatrizoate meglumine (hypaque-76, Winthrop Laboratories) was the radiopaque solution used for cerebral ventriculography. Beta-endorphin (Beckman) was prepared in sterile physiological saline to a volume of 5 μl for IVT injection.

Experimental Procedure. Rats were anesthetized with a mixture of ketamine (40 mg/kg) and xyalazine (10 mg/kg) injected intramuscularly. Slow infusion (2 μl/sec) of 0.25 ml of diatrizoate meglumine or air (0.25 ml) was administered through the IVT cannula. Specimen radiographs were obtained from film (XTL-2 film, Eastman Kodak) at an exposure of 43 kvp, 0.6 min at 30 in film to source distance (FTSD), using a cabinet x-ray system (Faxitron Series, Model 43805-N, Hewlett-Packard) immediately before and after each IVT injection.

Body temperature measurement. Body temperature was measured before and after radiography to evaluate the effectiveness of this technique for injecting beta-endorphin (B-END). Body temperature measurements were conducted on unrestrained rats placed in standard rodent shoebox cages in the controlled environment room. After a 30 min control period, rats were injected IVT with B-END and briefly removed from the cages at 0.5 hr intervals for measurement of rectal temperature (Tr) over 2 hr.

The procedure for measuring rectal temperature was described in Chapter 1.

Statistical analysis. Differences between treatments were determined by analysis of variance. Significance was assumed when $P < 0.05$.

Results

Intracerebroventricular injections of diatrizoate meglumine resulted in specimen radiographs showing movement of the radiopaque contrast material from the right lateral ventricle to the spinal column. Mixture of the contrast media with cerebrospinal fluid indicated correct positioning of the cannula in the right lateral ventricle (Figure C-1). Of the eight rats injected, seven recovered; resulting in an 88% survival rate. Air also was used as a contrast medium but specimen radiographs could not detect movement of the air through the ventricular system.

Rats injected IVT with 10 μg of B-END significantly ($P < 0.05$) elevated Tr (38.4 ± 0.14, $n = 8$) compared to saline-treated rats

Figure C-1. Specimen radiographs showing placement of intracerebroventricular cannula before (A) and after (B) injection of fluorescent dye.

A

B

(37.2 ± 0.20, n = 8) 60 min post-injection. Repetition of these Tr measurements after cerebral ventriculography produced similar results.

While diatrizoate melgumine is not lethal, three of the injected rats displayed minor convulsions. These convulsions began several min post-injection and, in the case of one rat, lasted for 2 hr. However, by the time the rats recovered from the anesthesia this response was not apparent. Also, on recovery, no changes in the behavior or appearance of these rats were observed.

Discussion

Oliver and Conrad (1975) and Peterson (1975) reported that air was the preferred contrast agent for myelography because this gas is completely absorbed and does not produce any long term side effects. In the present experiment, cerebral ventriculography using air as the contrast media did not result in radiographs that detected IVT cannula placement. This may have been due to the lack of constant pressure application when using air.

However, diatrizoate meglumine provided the best radiographic results. This technique produced clearly visible specimen radiographs (Figure C-1) that indicated correct placement of IVT cannula. The high survival rate (88%) and the lack of observed behavioral or other physical changes indicates that cerebral ventriculography is a valuable technique for verification of IVT cannula placement. These findings indicate that diatrizoate meglumine is a non-hazardous radiocontrast substance that is easily and safely handled. Moreover, this technique can serve as a useful tool in teaching this surgical procedure to others.

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BIOGRAPHICAL SKETCH

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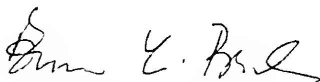

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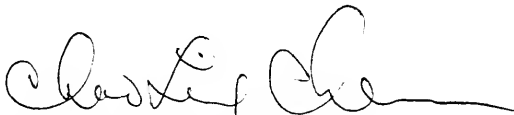

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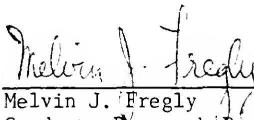

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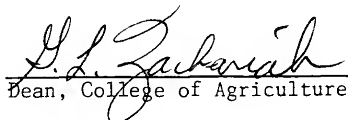
This thesis was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

April, 1984



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