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Adrenal and Thyroid Interactions of β-Endorphin-Induced Body Temperature Responses of Rats at 24.5°C[1] (42025)

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Abstract. The effect of β-endorphin (β-END) and the role of the adrenal and thyroid glands on body temperature were examined in male rats in a controlled environment room at 24.5 ± 0.1°C. Relative humidity of 50 ± 0.3% and a 12L:12D photoperiod (L = 0900 to 2100 hr) were maintained. Rectal temperature (T_r) was measured using thermistors. Corticosterone and thyroid hormones were determined by radioimmunoassay. Intracerebroventricular (IVT) administration of varying doses (0.05 to 50.0 μg) of β-END resulted in a hyperthermia that began 30 min post-IVT injection and continued for an additional hour. Intravenous injections of the same doses of β-END resulted in little or no T_r response. The β-END-induced hyperthermia was antagonized by intraperitoneal injection of naloxone. Pretreatment with propranolol, phenotolamine, or both drugs in combination did not block the hyperthermia caused by β-END. Adrenalectomized or hypophysectomized rats receiving IVT injections of β-END did not consistently display an increased T_r. β-Endorphin administration had no detectable effect on serum corticosterone or thyroxine but serum triiodothyronine was decreased. These data suggest the acute hyperthermic action of β-END is mediated centrally through opiate receptors and does not involve adrenergic receptors.

It has been reported that both physiological levels and low doses of β-endorphin (β-END) produce hyperthermia (1), but high doses produce either hypothermia or hypothermia followed by a hyperthermic (biphasic) response (2). Although β-END does not readily cross the blood–brain barrier (3), intravenous administration produces a slight hyperthermia in rats at 23 to 25°C (4). Intracerebroventricular (IVT) injections of β-END cause both thermal and analgesic responses which are dependent on the administered dose (5).

Naloxone appears to block (2, 6, 7) or antagonize (8) the body temperature response to β-END, while in other experiments naloxone appears to have no effect on body temperature (9). Naloxone antagonism of opioid hypothermia (7) and hyperthermia (2) has been reported. Alternatively, naloxone antagonism of the temperature response to β-END may be dose related (10).

The present study was conducted to clarify the relationship between β-END and body temperature in the rat adapted to a thermoneutral temperature of 24.5°C. The hypothesis that β-END may alter body temperature through calorigenic hormones from the adrenal and thyroid glands also was examined.

Materials and Methods. *Animals.* Adult, male Sprague–Dawley rats [Caw:CFE (SD)], weighing 350.5 ± 6.2 g (mean ± SE) were used in these experiments. Rats were housed individually, in metabolic cages (Model 4-461-00, 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 beginning each experiment. Water bottles equipped with sipper tubes were attached to each cage. Food (Laboratory Rodent Chow, Ralston Purina Co.) and water were available *ad libitum.*

Intracerebroventricular (IVT) cannulae. At least 72 hr before experiments began, an intracerebroventricular stainless steel cannula was implanted stereotaxically[2] into the right

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lateral ventricle while each rat was under ether anesthesia. Cannula placement was according to the modified de Groot system (11). Correct positioning of each cannula was initially verified by the pulsatile flow of cerebrospinal fluid and further verification was obtained at necropsy and by cerebral ventriculography (12).

Intravenous cannulae. Under ether anesthesia, an iv cannula was implanted into the right jugular vein of each rat (13). A recovery period of at least 24 hr was allowed before experiments began. 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 sampling box. The top of the sampling box was equipped with an opening for easy access to the iv cannula. The box allowed free movement of the animal and was equal in size to a standard rodent shoebox cage.

Drugs. Varying doses of β-END (0.05 to 50 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),[3] propranolol (Sigma Chemicals), and phentolamine (Ciba-Geigy Pharmaceuticals)[3] were injected intraperitoneally. Ampicillin (Bristol Laboratories) and procaine penicillin-G (Pfizer) were used postoperatively to prevent infection.

Adrenalectomized rats. Adrenalectomy[2] was performed under ether anesthesia through the bilateral paralumbar approach (14). Following surgery, rats were maintained on a 0.9% NaCl drinking solution, available *ad libitum*. A 1-week recovery period was allowed before beginning experiments. Completeness of adrenalectomy was verified by trace serum corticosterone levels and at necropsy.

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 and at necropsy by examining the sella turcica for pituitary tissue.

Radioimmunoassays (RIA). Serum corticosterone levels were measured by radioimmunoassay (15). Measurements of serum thyroid hormones were made using commercially available RIA kits (Clinical Assays).

Body temperatures. Rectal temperature (T_r) was measured using a thermistor (Model 402, Yellow Springs Instruments) inserted 5 cm into the rectum and held in place with surgical tape wrapped around the tail. The thermistor was connected to a telethermometer (Model 44 TD, Yellow Springs Instruments) for visual display of the temperature. Changes in rectal temperatures were computed as the differences between the beginning (0 time) and ending (minutes postinjection) values of the T_r during each experimental session.

Experimental procedure. Experiments were conducted on unrestrained rats placed in standard rodent shoebox cages in a controlled environment room at 24.5°C. This ambient temperature is considered to be an acceptable "control" temperature for β-END research (16) and lies in the middle of the thermoneutral zone for rats (17). After a 30-min control period, rats were injected IVT and briefly removed from the cages, at 30-min intervals, for measurement of rectal temperature over a 3-hr period. The rectal temperatures of rats pretreated with propranolol and phentolamine were measured every 15 min for the first hour followed by 30-min measurements for an additional 2 hr. To accustom rats to handling, all rats were weighed three times a day for 2 weeks before beginning each experiment. All measurements or sample collections were performed between 1500 and 1800 hr.

Statistical analysis. Analysis of variance was used to determine differences within each treatment group and between treatments. Differences between means were determined by Duncan's multiple range test. In all cases, significance was assumed when $P < 0.05$.

University, Boston, Mass., for their assistance in adrenalectomizing the rats and developing the intracerebroventricular cannula procedure used in this study.

[3] The generous donation of naloxone by Endo Laboratories, Garden City, N.Y., and phentolamine by Ciba-Geigy Pharmaceuticals, Summit, N.J., is greatly appreciated.

Results. For rats receiving IVT injections of β-END, the T_r was highly significantly elevated ($P < 0.01$) above the level of rats receiving iv injections at all doses between 5.0 and 50.0 μg (Fig. 1). For the IVT injected group, rectal temperature remained elevated for at least 90 min, then declined and returned to control values within 180 min postinjection. Because the maximal T_r response occurred 60 min postinjection, this time interval was selected to compare all subsequent measurements.

The hyperthermia produced by IVT injections of β-END was antagonized by subsequent intraperitoneal injection (1 mg/kg) of naloxone (Fig. 2). This response was rapid, and T_r returned to control levels within 15 min following naloxone injection. Administration of saline following the IVT injection of β-END did not alter the hyperthermia. Rats receiving IVT injected saline followed by naloxone did not display significant changes in T_r.

Intracerebroventricular injections of varying doses (0.05 and 50.0 μg) of β-END into adrenalectomized and hypophysectomized rats generally resulted in an increased T_r but not to the same level as observed in intact rats (Fig. 3). β-Endorphin doses between 10 and 50 μg resulted in significantly lower

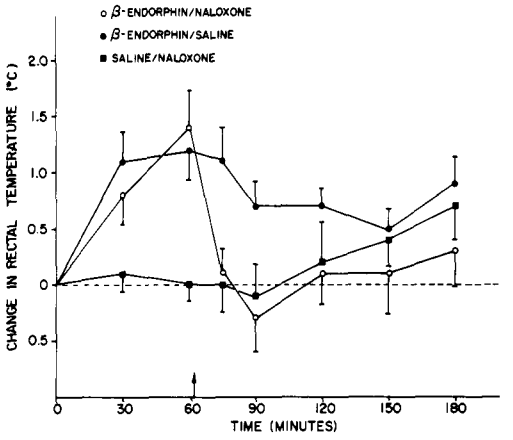

FIG. 2. Temporal effects on rectal temperature of β-endorphin followed by saline, β-endorphin followed by naloxone, and saline followed by naloxone on rectal temperature changes of rats at 24.5°C. Intracerebroventricular injection of β-endorphin (10 μg) or saline was made at 0 time. The arrow represents intraperitoneal administration of naloxone (1 mg/kg) or saline. Each point represents the mean and the vertical bars the standard errors for 10 rats.

increases in T_r of adrenalectomized compared to intact rats. For hypophysectomized rats, lowered ($P < 0.01$) T_r were observed only at doses of 5, 10, and 50 μg. Significant dose-

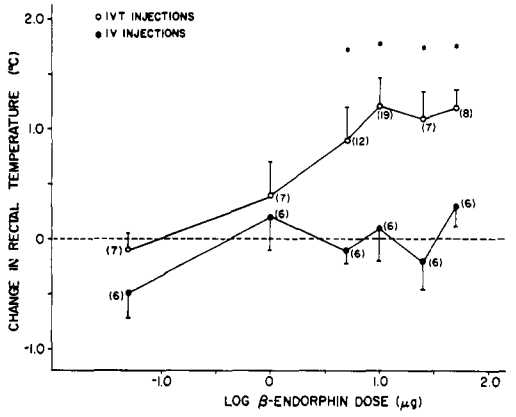

FIG. 1. Comparison of effect of intravenous (IV) and intracerebroventricular (IVT) injections of varying doses of β-endorphin on body temperature changes 60 min postinjection. Each point represents the mean value and the vertical bars the standard errors. Asterisks indicate significant ($P < 0.05$) differences between groups and the values in parentheses indicate the number of rats used.

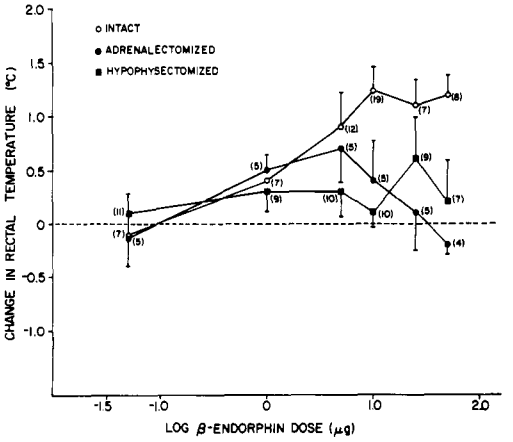

FIG. 3. Changes in rectal temperature for intact, adrenalectomized, and hypophysectomized rats receiving intracerebroventricular injections of varying doses of β-endorphin. Each point represents the mean value and the vertical bars the standard errors. Values in parentheses indicate the number of rats used.

TABLE I. SERUM CORTICOSTERONE AND RECTAL TEMPERATURE (T_r) CHANGES FOR SIX RATS BEFORE AND AFTER INTRACEREBROVENTRICULAR INJECTION OF β-ENDORPHIN

Dose of β-END (μg)	Serum corticosterone (μg/dl)			Change in T_r[a] (°C)
	Before β-END injection	After β-END injection	Change	
0 (saline; no β-END)	18.2 ± 0.5	22.0 ± 0.3	3.8	0.0
1.0	17.7 ± 1.7	22.8 ± 0.7	5.1	0.3 ± 0.4*[b]
10.0	17.0 ± 0.7	23.3 ± 0.9	6.3	1.2 ± 0.2**
25.0	17.3 ± 0.8	23.8 ± 1.2	6.5	1.3 ± 0.3**

[a] Change in rectal temperature equals 60 min postinjection value minus 0 time value and expressed as means ± SE.

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

related changes in serum corticosterone (Table I) were not detected in rats receiving IVT injections of varying doses of β-END; however, serum triiodothyronine (Table II) decreased.

Intraperitoneal injections of propranolol (6 mg/kg), phentolamine (6 mg/kg) or simultaneous administration of both drugs decreased T_r below that of saline (zero) controls (Fig. 4). Propranolol caused the least decrease in T_r. Rats administered phentolamine or both drugs (propranolol and phentolamine) in combination displayed a further decreased T_r. Rectal temperature remained lowered until at least 90 min postinjection and began to increase within 120 min.

Intracerebroventricular administration of β-END to rats about 30 min after treatment with propranolol, phentolamine, or propranolol and phentolamine together increased T_r (Fig. 5). In the saline, propranolol, and phentolamine groups, the increase in T_r was about 0.9°C and began about 30 min following injection of these substances. For the propranolol and phentolamine group, the increase in T_r also began about 30 min following injection but was about 50% greater than when propranolol or phentolamine were injected individually.

Discussion. In previous reports on the effects of opioids on body temperature in rats, three major patterns appear: hyperthermia (1), hypothermia, or a biphasic response (2). These different responses may have resulted from the range (0.6 μg to 0.5 mg) of doses and the routes (ip, IVT, iv) of administration used in those studies (5). On the other hand, the low doses of β-END (0.05 to 50.0 μg) used in the present study produced hyperthermic responses only when injected

TABLE II. SERUM TRIIODOTHYRONINE (T_3) AND THYROXINE (T_4) LEVELS, AND RECTAL TEMPERATURE (T_r) CHANGES OF SIX RATS BEFORE AND AFTER INTRACEREBROVENTRICULAR INJECTION OF β-ENDORPHIN

Dose of β-END (μg)	T_3 (ng/dl)			T_4 (μg/dl)			Change in T_r[a] (°C)
	Before β-END injection	After β-END injection	Change in T_3	Before β-END injection	After β-END injection	Change in T_4	
0 (saline)	34.8 ± 3.7	35.4 ± 4.3	0.68	2.2 ± 0.2	1.9 ± 0.2	−0.2	0.0
1.0	33.5 ± 5.2	25.6 ± 2.0	−7.9*[b]	2.2 ± 0.3	2.2 ± 0.3	0.1	0.4 ± 0.1*
10.0	32.7 ± 2.3	22.2 ± 2.1	−10.5*	2.4 ± 0.2	2.3 ± 0.3	−0.3	1.1 ± 0.1**
25.0	24.6 ± 1.7	20.3 ± 1.5	−4.3*	2.6 ± 0.3	2.3 ± 0.2	−0.2	1.2 ± 0.3**

[a] Change in rectal temperature equals 60 min postinjection value minus 0 time measurement and expressed as mean ± SE.

[b] Asterisks denote significant ($P < 0.05$) differences within each group as determined by analysis of variance and Duncan's multiple range test.

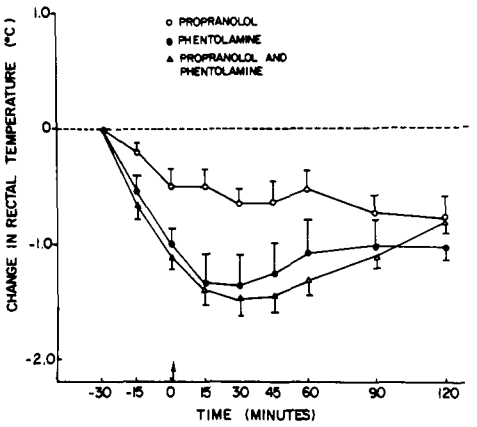

FIG. 4. Change in rectal temperature for rats receiving an intraperitoneal injection of propranolol, phentolamine, or propranolol and phentolamine prior to intracerebroventricular injection of 10 μl of saline (arrow). Each point represents the mean and the vertical bars the standard errors for six rats.

IVT. Intravenous administration of the same doses did not significantly elevate T_r which is consistent with previous findings (3) that only a small percentage (0.3%) of iv injected B-END crosses the blood–brain barrier. The current findings strongly suggest that the hyperthermia resulting from β-END injected IVT is mediated through a central mechanism.

β-Endorphin may mediate changes in body temperature through central thermoreceptors or through calorigenic or vasoactive hormones (18). These actions may involve β-END alone or in combination with one or more neurotransmitters and may depend on the dose of β-END. Opiate effects may result from low doses of β-END while nonopiate effects, which cannot be antagonized by naloxone, may result from higher doses (5).

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

The antagonistic effect of naloxone on the β-END caused hyperthermia (Fig. 2) previously has been reported (6) and suggests that naloxone sensitive receptors may be involved. Others have found β-END-induced hyperthermia to be resistant (19) or partially resistant (20) to naloxone antagonism. It is known that naloxone strongly binds to μ receptors but is less dominant at other opioid receptors (21).

Neither adrenalectomized nor hypophysectomized rats injected IVT with β-END demonstrated changes in rectal temperature comparable to that produced by intact rats (Fig. 3) at any dose of β-END or time interval studied. At doses of β-END above 5 μg, adrenalectomized rats displayed lower rectal temperature changes compared to intact rats. This altered responsiveness may indicate an increased sensitivity to the dose of β-END administered and is consistent with previous studies (22). Hypophysectomized rats had reduced plasma (23) and brain (24) β-END-like-immunoreactivity which may have altered the sensitivity of opiate receptors. Adrenal atrophy, a consequence of hypophysectomy, may have altered the hormone balance necessary to produce the T_r effects of β-END.

Previous studies suggest adrenocortical involvement in opiate-induced hyperthermia (25) in which serum corticosterone may have a permissive role (16). In the study reported herein, serum corticosterone levels were elevated by β-END but they were not significantly different from saline controls (0 dose of β-END). Positive correlations between

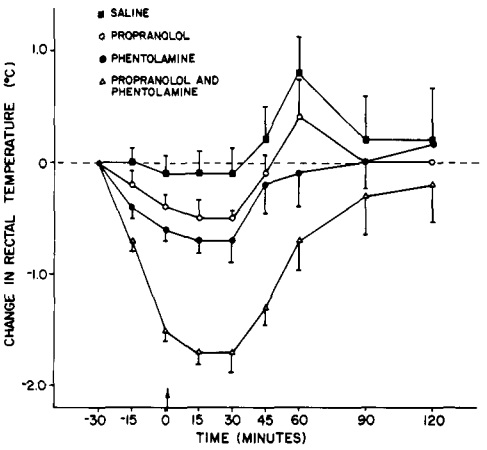

FIG. 5. Temporal effects resulting from pretreatment of rats with saline, propranolol, phentolamine, or propranolol and phentolamine prior to intracerebroventricular administration of 10 μg of β-endorphin (arrow). Each point represents the mean value and the vertical bars the standard errors for six rats.

plasma levels of β-END and ACTH, but not cortisol, have been reported in humans (26). Those authors attributed this response to different adrenal receptor sensitivities to ACTH or differences in metabolic clearance rates of plasma ACTH and cortisol. Their data may reflect concomitant releases of ACTH and β-END from the pituitary gland. On the other hand, β-END and ACTH may act in opposition in the maintenance of body temperature (25).

Thyroid hormones may modify endogenous levels of β-END in brain tissues (27) and present findings suggest thyroid hormone involvement as evidenced by the decreased serum T_3 levels in rats receiving β-END. In contrast, thyrotropin releasing hormone has been reported to reverse β-END-induced hypothermia in rats (7).

Body temperature also may be influenced by the catecholamines, epinephrine and norepinephrine (28). Blockage of adrenergic receptors with propranolol, phentolamine, or phentolamine and propranolol in combination consistently decreased T_r. Body temperature appeared to be lowered because of tail vasodilation due to loss of vasoconstrictor tone (α-receptor mediated) or decreased metabolic rate (predominantly β-receptor mediated). Nevertheless, when β-END was administered IVT to all of these groups, the T_r was increased (Fig. 5), suggesting that β-END-induced hyperthermia is not mediated through adrenergic receptors. Alternatively, β-END may increase T_r by removing these blockers from the appropriate receptor site; however, other evidence (28) suggests that this is unlikely.

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