Correction. In the article "Endorphins may function in heat adaptation" by John W. Holaday, Eddie Wei, Horace H. Loh, and Choh Hao Li, which appeared in the June 1978 issue of the Proc. Natl. Acad. Sci. USA (75, 2923–2927), the following undetected printer’s error occurred. In the right-hand column of p. 2926, the first paragraph should read: "In other work, we have shown that hypophysectomy alters responses to injected opiates (15). From these studies, we conclude that the absence of the pituitary may modify the function of endorphins in response to heat. It is possible that circulating endorphins of pituitary origin may gain access to the central nervous system through brain areas devoid of a blood–brain barrier—i.e., the subfornical region or area postrema (22). In this regard, it has been shown that intravenously injected β-endorphin is 3 times more potent than morphine on a molar basis in producing antinociception in mice (15, 23). Alternatively, recent studies have supplied anatomical evidence that the anterior and posterior pituitary may directly secrete to the brain via the vasculature from the infundibular area to the third ventricle and hypothalamus (24, 25). This, too, may result in a pituitary endorphin modulation of central nervous system function.”

Correction. In the article "Local mutagenesis: A method for generating viral mutants with base substitutions in preselected regions of the viral genome" by David Shortle and Daniel Nathans, which appeared in the May 1978 issue of Proc. Natl. Acad. Sci. USA (75, 2170–2174), the Bgl I cleavage sites shown in Fig. 2 were incorrectly drawn. The sites deduced by B. S. Zain and R. J. Roberts (personal communication) are shown by arrows in the following nucleotide sequence:

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GGCCGAGGC GCCCTCGGCC
CGGCTCCGC CGAGCGG
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↓

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GGCCGAGGC GCCCTCGGCC
CGGCTCCGC CGAGCGG
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Endorphins may function in heat adaptation  
(thermoregulation/hypophysectomy/naloxone/heat)

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Contributed by Choh Hao Li, March 6, 1978

ABSTRACT Administration of the opiate antagonist naloxone to rats after acute or chronic heat exposure precipitates an increase in colonic temperature, an increase in escape attempts, and a decrease in body weight. These changes are accompanied by signs associated with hyperthermia, such as salivation, diarrhea, and an abnormal extended posture. Although brain endorphin involvement is possible, hypophysectomy diminishes the intensity and magnitude of these naloxone effects, indicating that the naloxone effect in intact animals may be due to a functional antagonism of pituitary endorphins. These observations suggest that endorphins attenuate physiological responses to thermal and noxious stimuli triggered in common neuroanatomical pathways by heat.

Subsequent to the discovery of endogenous opiate-like peptides (endorphins) (see refs. 1 and 2 for review), a primary research objective has been to elucidate the functional role of these substances in the body. In the absence of sensitive and specific chemical assays for these compounds, investigators have used the "pure" opiate antagonist naloxone in attempts to block physiological endorphin effects and thereby to infer a role for endorphin in various behaviors. With regard to responses to noxious stimuli, such investigations have yielded contradictory evidence for endorphin involvement (3–5).

Because administration of alkaloid opioids and endorphins has been shown to alter body temperature (6–8), it is of interest to assess the possible role of naloxone in this regard. Goldstein and Lowery (9) were unable to show an effect of naloxone on animals subjected to cold stress; however, Lal and coworkers (10) were able to reverse conditioned hyperthermia with naloxone.

Since higher doses of opiates generally produce hyperthermia (6, 11–13), we propose that endorphin systems may be physiological determinants in responses to heat, not cold. Our studies reported herein show that acute and chronic exposure to elevated temperatures and subsequent naloxone administration elicited a rapid increase in colonic temperature accompanied by an increase in attempts to escape from a confining enclosure and a decrease in body weight. Furthermore, hypophysectomy attenuated these responses to naloxone in both acutely and chronically heat-exposed rats.

MATERIALS AND METHODS

In the first study, male Sprague–Dawley rats weighing 260–300 g were employed because of previous use of this strain of rats in evaluating precipitated opiate withdrawal effects at various ambient temperatures (14). Colonic temperatures were measured in the room where the animals were housed (23.0° ± 0.5°) by means of a thermistor probe inserted rectally to a depth of 6 cm. Rats were then placed in glass jars within a hot room (average temperature 36.6°, 20–30% humidity) for 1 hr. Physiological saline or naloxone in saline (10 mg/kg, Endo Laboratories, Garden City, NY) was injected intraperitoneally (0.1 ml/100 g body weight) in a blinded fashion. The escape attempts from the jars were counted in 5-min intervals for a total of 15 min after injections. Colonic temperatures were again measured 45 min after injections.

In the second study, designed to assess the effects of hypophysectomy on thermoregulatory behaviors in response to naloxone, male Long–Evans rats (180–220 g) were surgically prepared and were maintained on food and water ad lib at an ambient temperature of 26.5° ± 0.5° for at least 1 wk after surgery as described (14). For the acute experiment, rats were placed within jars in the hot room (37.0° ± 0.5°, 20–30% humidity) for 1 hr. In pilot studies, it was determined that chronic exposure of rats to temperatures ≥35.0° killed several of the sham-operated animals. Therefore, for chronic heat exposure experiments, rats were kept at 34.5° ± 0.5° (20–30% humidity). After measurement of body weight and colonic temperature, each rat was sham injected and escape attempts prior to injection of naloxone were enumerated. Subsequently, naloxone (10 mg/kg) was injected as before; escape attempts were enumerated for 10 min and scored during two 5-min intervals. At 20–30 min after naloxone administration, body weights and colonic temperatures were again measured in both acutely and chronically heat-exposed rats. Temperatures depicted on figures are averages obtained over the course of each study.

Statistical significance for percent weight loss, as well as for change in colonic temperature, was evaluated by Student’s t test. In Fig. 1, escape attempts were statistically evaluated by the Mann–Whitney U test; one-tailed statistics were employed where results were anticipated (blinded study). In comparisons of pre- and post-naloxone effects in hypophysectomized and sham-operated control rats (Figs. 2 and 3), the Wilcoxon matched-pair, signed-ranks test was used. Linear regressions were obtained by the method of least-squares. Statistical significance was ascribed if P < 0.05 unless otherwise noted.

RESULTS

Acute exposure of normal rats to an ambient temperature of 36.6° (1 hr) resulted in an average colonic temperature increase of 1.73°. After injections, colonic temperatures of saline-injected control rats significantly decreased over the ensuing 45 min by 0.41° (paired t, P < 0.02, df = 7). However, 45 min after naloxone injection there was no decrease but instead a slight increase in colonic temperature (0.08°).

As can be seen in Fig. 1, in rats acutely exposed to 36.6°, there was a progressive increase in the average number of escape

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attempts over time for the naloxone-injected rats whereas saline-injected controls maintained the same baseline escape frequency. During the 10–15 min post-drug interval, the number of escape attempts for the naloxone-injected group was significantly greater than that for the saline group ($P < 0.05$, one-tailed, $n = 16$). Injection of naloxone at neutral environmental temperatures did not cause escape attempts or acute weight loss.

At the usual maintenance temperature of 26.5°C, hypophysectomized rats have a lower colonic temperature than sham-operated controls (Fig. 2). However, comparison of colonic temperatures after 1-hr exposure to 37.3°C revealed that both groups attained the same elevated temperature (≈41.3°C). Heat alone produced a significant decrease in body weight. Superimposed upon the effect of heat, subsequent naloxone administration in sham-operated controls resulted in an additional decrease in body weight ($P < 0.001$, df = 5), an increase in number of escape attempts ($P < 0.025$, $n = 6$), and an increase in colonic temperature when measured 20 min after injections ($P < 0.01$, df = 5). By contrast, in spite of the fact that the same pre-drug colonic temperatures were attained by hypophysectomized and control rats in the hot room, naloxone administration in hypophysectomized animals did not significantly increase colonic temperature at 20 min or escape attempts; however, weight loss after naloxone was significant ($P < 0.001$, df = 5).

Unlike acutely heated rats, in which colonic temperatures of control and hypophysectomized animals were the same after 1 hr at 37.3°C, chronically heat-exposed hypophysectomized rats (2 days at 34.5°C) were about 1.3°C colder than controls (Fig. 3). Otherwise, the differences between the effects of acute and chronic heat exposure were minimal. Naloxone injections in these chronically heat-exposed rats resulted in the same general pattern of changes in body weight, colonic temperature, and escape attempts as occurred in acute studies (compare Figs. 2 and 3). As before, exposure to heat alone produced a weight loss that was further potentiated by naloxone treatment. The increase in colonic temperature was significant 30 min after naloxone injection in both control and hypophysectomized rats; however, as with acute studies, the temperature increase was greater in sham-operated control rats. Escape attempts were likewise greater in control animals; however, statistical significance was only obtained 5–10 min after naloxone injection in the control group. Escape behavior was almost never observed in the hypophysectomized group.

In addition to the three types of naloxone-induced effects reported above, naloxone-injected control rats, in both acute and chronic studies, exhibited an enhancement of saliva-spreading behavior as well as an increase in diarrhea and in abnormal posturing. Hypophysectomized animals were not observed to salivate or to demonstrate the abnormal, spread

FIG. 1. Effects of naloxone (10 mg/kg) on escape attempts in rats acutely exposed to heat (36.6°C). Shaded areas represent naloxone-injected (intraperitoneally) rats ($n = 8$); clear areas represent saline-injected animals ($n = 8$). Vertical lines delimit SEM. Asterisk denotes significant increase ($P < 0.05$, one-tail) in number of escape attempts as compared to saline-injected controls.

FIG. 2. Hypophysectomized rats (darker area) are compared with sham-operated control rats (clear area) in responses before and after acute heat exposure (1 hr, 37.3°C) and subsequent naloxone (10 mg/kg) injection (nalox). Vertical lines represent SEM. Comparisons are made only within surgical groups ($n = 6$/group). Asterisk denotes $P < 0.05$; triangle indicates no significant differences. All effects occurred by 20 min after injection.
posture after naloxone injection; however, mild diarrhea did occur in those animals. Wet-dog shakes and teeth-chattering were seldom observed after drug injections in either group of animals.

The interrelationship among increases in body temperature, escape attempts, and loss in body weight are shown in Fig. 4. For sham-operated control animals exposed to 34.5°C for 2 days, the naloxone-induced increases in colonic temperatures were positively correlated with increases in escape attempts ($r = 0.92$, $P < 0.05$) and decreases in body weight ($r = 0.97$, $P < 0.02$). Although naloxone significantly increased colonic temperature and decreased body weight in hypophysectomized animals, no significant increase in escape attempts was demonstrated. Furthermore, there were no correlated relationships among these measures in hypophysectomized rats.

**DISCUSSION**

In prior work, we have shown that intraventricular injections of relatively low doses of $\beta$-endorphin produce a mild hyperthermia (15). In addition, $\beta$-endorphin-induced wet-dog shakes, presumably a heat-generating behavior, were observed to be correlated with increases in colonic temperature (16). After cessation of wet-dog shakes, a clonic seizure-like state accompanied by copious salivation—a heat-loss behavior (17)—was observed to occur (16). Ultimately, however, higher doses of opiates invariably produced hypothermia in the drug-naive rat (6, 16). The purpose of these studies, therefore, was to test the hypothesis that endorphins may subserve a role in thermoregulation.

The observation that acute heat exposure alone (1 hr) increases colonic temperatures was made in both acute experiments. In the first study, injections of saline at 1 hr in these control animals was followed by a significant decline in colonic temperature 45 min later. However, in rats that were injected with naloxone, this adaptation to the hot environment over the ensuing 45 min was blocked, thus implicating endorphin involvement in acute heat adaptation. In chronically heat-exposed rats, colonic temperatures of sham-operated controls or hypophysectomized rats were not significantly different from pre-exposure values. This would indicate an adaptation to this heated environment over the 2 days of exposure. However, as with acute heat exposure, in chronically heated rats naloxone again caused the body temperature of the sham-operated control animals to increase and was without effect in the hypophysectomized group.

The results of these studies show an effect of naloxone on acutely and chronically heat-exposed animals. Insofar as naloxone may be used as an inferential tool to examine the physiological significance of endorphin systems, we believe that these data support the hypothesis that endorphins are involved in adaptation to heat and therefore in maintenance of normothermia. In order to corroborate this relationship, one may ask: Is the naloxone effect on these measurements an indicator of endorphin involvement?

In response to this question, it is recalled that administration of pharmacological doses of opiate alkaloids or endorphins alters body temperature (6-8, 16). Furthermore, naloxone, a relatively "pure" narcotic antagonist, not only blocks acute opioid effects but also precipitates abrupt withdrawal in opiate-dependent subjects. Classically, changes in body weight, temperature, and escape attempts are employed as indicators of such opiate dependence, whether spontaneously occurring after cessation of opiate administration or precipitated by naloxone injection (18, 19). Thus, if it occurs as a physiological condition, endorphin deficiency must mimic the signs of the opiate withdrawal syndrome and it may be inferred that naloxone-induced alterations of these measures in heat-stressed animals portends

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**Fig. 3.** The effects of naloxone (10 mg/kg) on chronically heat-exposed rats (2 days, 34.5°C). Hypophysectomized rats ($n = 6$) are indicated by shaded areas; clear areas represent sham-operated control animals ($n = 5$). Values represent mean ± SEM; asterisk denotes $P < 0.05$; triangle indicates a lack of statistical significance. Escape attempts were enumerated over a 10-min post-injection time span; weight loss and colonic temperature changes were measured by 30 min after injection.
lower than that of controls, and the lack of correlated changes may relate to the differences in these body temperature baselines.

In other work, we have shown that hypophysectomy alters responses to injected opiates (15). From these studies, we conclude that the absence of the pituitary may modify the function of endorphins in response to heat. It is possible that circulating endorphin is 3 times more potent than morphine on a molar nervous system through brain areas devoid of a blood–brain barrier—i.e., the subfornical region or area postrema (22). In this regard, it has been shown that intravenously injected β-endorphin is 3 times more potent than morphine on a molar basis in producing antinociception in mice (23, 15). Alternatively, recent studies have supplied anatomical evidence that the anterior and posterior pituitary may directly secrete to the brain via the vasculature from the infundibular area to the third ventricle and hypothalamus (24, 25). This, too, may result in a pituitary endorphin modulation of central nervous system function.

It has been reported that morphine may depress the hypothalamic thermoregulatory set point (26) or elevate this thermostat set point (27), depending upon the dose. Others have shown mild hyperthermia to occur in response to injections of nalorphine (26, 28) or the more pure opiate antagonist, naloxone (27), at neutral environmental temperatures. However, Goldstein and Lowery demonstrated no effect of naloxone in cold-exposed rats (2", 16 hr; table 1 of ref. 9). By contrast, we found a profound hyperthermia in response to naloxone injections in acutely and chronically heated rats (Figs. 2 and 3). In fact, the temperatures attained under these conditions have been shown to cause death in rats (29). Thus, we suggest that endorphin systems appear to be selectively activated in response to heat stress, not cold stress. We suggest that exogenously administered opiates and/or physiologically functioning endorphins may result in a "sensed" hypothermia. With low doses, the "sensed" lowering of the thermoregulatory set-point results in compensatory thermogenic activity (i.e., wet-dog shakes and escape attempts). The result is an actual increase in body temperature. In fact, we have shown that the wet-dog shakes that others have reported to result from intraventricular β-endorphin injections (8) are also correlated with increases in colonic temperature (16). Since higher doses of opiates produce a more immobilized animal progressing to a state of catalepsy (8, 16), the animal would be unable to elicit thermogenic behavior and an actual decline in colonic temperature would ensue.

In support of the hypothesis that endorphins may serve as physiological attenuators of sensors for heat-gain mechanisms in the body, it would be expected that morphine-tolerant rats would be tolerant to the tonic hypothermic effects of endogenous opiates as well. Indeed, Gunne (12) reported that the body temperature of tolerant rats become significantly elevated. Moreover, it has also been reported (30) that morphine-tolerant rats that are accidentally exposed to heat are unable to adapt and therefore die.

In summary, we suggest that endorphins may function in adaptive mechanisms to heat. Furthermore, the data indicate that pituitary endorphins may be involved in the manifestations of these effects. In a prior report, we postulated that an interplay between thyrotropin-releasing hormone and endorphins may exert a dynamic effect on maintenance of normothermia (31). Because of the antinociceptive effects of pharmacologically administered opiates, it has been assumed that a primary function of endorphins is to modulate the perception of pain. Since the perception of both pain and temperature sensations are mediated through some common neuroanatomical path-

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**Fig. 4.** Comparisons between increased colonic temperature and escape attempts (X and dashed line) and increased temperature and percent weight loss (solid line) in chronically heated rats (2 days, 34.5°C). These events are correlated in sham-operated control animals (P < 0.02), whereas no such correlation was found in hypophysectomized rats.
ways, it seems likely that endorphins modulate temperature responses in addition to other nociceptive behaviors.

We thank Ms. Barbara Hitzemann, Mr. J. D. Nelson, Mr. J. B. Cunningham, and Ms Judy Quinn for their expert assistance. This work was supported in part by Walter Reed Army Institute of Research, National Institute of Drug Abuse Grants DA-00564 and DA-00091, and National Institute of Mental Health Grant MH-30245.
