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The Role of Endogenous Opioids in Footshock-Induced Hyperthermia[1]

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PECHNICK, R. N. AND M. J. MORGAN. *The role of endogenous opioids in footshock-induced hyperthermia.* PHARMACOL BIOCHEM BEHAV **28**(1) 95–100, 1987.—Either opioid or nonopioid forms of stress-induced analgesia can be elicited depending on the intensity or duration of the stressor. Several forms of stress have also been shown to cause hyperthermia in the rat. The present study investigated whether opioid and nonopioid forms of such stress-induced hyperthermia can be elicited in the rat as a function of footshock current intensity. Rats were given footshock after pretreatment with saline or naltrexone, or chronic morphinization. Footshock produced hyperthermia, the degree of which was found to be a function of current intensity. While the peak rise in temperature was not affected by naltrexone or chronic morphine administration, the rate of return to baseline temperature was slowed by these treatments. Thus, the endogenous opioid system appears to be involved in the return to normal body temperature following footshock, but not in the footshock-induced rise in temperature.

Hyperthermia Thermoregulation Temperature Opioids Opiates Morphine-tolerance
Naltrexone Opiate antagonists Stress Rat

VARIOUS stressors such as handling, restraint, exposure to a novel environment, and footshock are known to cause increases in body temperature in the rat [5, 19, 26, 29]. Such stressors are also known to cause the release of opioid peptides [1, 2, 21, 22], and produce alterations in opiate receptor occupancy [23]. Because exogenously administered opiates and opioid peptides produce profound changes in body temperature [6,7], it has been hypothesized that stress-induced hyperthermia may be mediated by endogenous opioid peptides [2,26]. In support of this hypothesis it has been reported that stress-induced hyperthermia can be antagonized by pretreatment with opiate antagonists [2, 19, 26].

Stressors can also cause potent analgesia in the rat [1, 4, 11, 17, 30]. It has been reported that varying the intensity or duration of continuous footshock can elicit analgesia involving different neurochemical substrates that can be classified as opioid or nonopioid in nature [27]. Thus, footshock of lower current intensity or of briefer duration elicits analgesia that meets several criteria for the involvement of opioid peptides (blocked by opiate antagonists, manifests tolerance with repetition and cross-tolerance with morphine); whereas footshock of higher current intensity or longer duration causes nonopioid analgesia by the same criteria.

The present study investigated whether opioid and nonopioid forms of stress-induced hyperthermia can be elicited in the rat as a function of footshock current intensity. The first experiment examined the relationship between footshock current intensity and changes in core temperature in order to ascertain if the degree of hyperthermia is a function of current intensity. The second experiment tested the involvement of endogenous opioids in shock-induced hyperthermia by determining the effect of pretreating the rats with the opiate antagonist naltrexone. The third experiment further assessed the opioid nature of footshock-induced hyperthermia by testing the hyperthermic response in morphine-tolerant subjects.

METHOD

Animals

Male Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA), weighing 350–450 g were used in all experiments. They were housed six to a cage under a 12-12 hr light-dark cycle (lights off 08:00–20:00 hr) for at least 7 days prior to the experiment. Standard rat chow and water were available ad lib. Subjects were only used for a single experiment.

Temperature Measurements

All experiments were performed between 11:00 and 16:00 hr. The temperature in the test room was held between 22.0–24.0°C. Core temperatures were measured by means of a telethermometer (Yellowsprings Instruments, Yellow Springs, OH). The rats were gently held while the thermistor probe was inserted 5 cm into the rectum. The probe was left in for approximately 30 sec until equilibrium was established.

Experiment 1

The animals were moved in their home cage into the test

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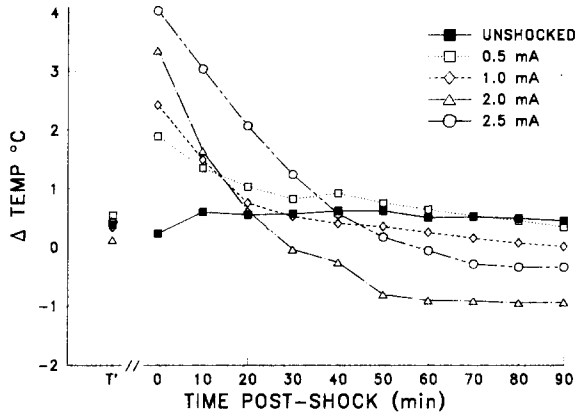

FIG. 1. The effects of handling and footshock current intensity on mean change in body temperature from baseline. T' is the change in temperature 15 min after the baseline temperature measurement and immediately prior to footshock. N=8 per group.

room 60 min prior to handling. The rats were then weighed, a baseline temperature recorded, and they were given an injection of 0.9% saline (1.0 ml/kg SC). They were then placed in a holding cage (30×30 cm) with sawdust on the floor. Post-injection temperatures were taken 15 min later in order to test the effect of handling, and the rats were moved into individual Plexiglas shock chambers (23×23×20 cm). Subjects were randomly assigned to treatment groups receiving either no shock or intermittent footshock (60 Hz, on 1 sec every 5 sec) delivered through a scrambler to the grid floor. Shocked subjects received constant current footshock of 0.5, 1.0, 2.0, or 2.5 mA for 20 min while unshocked subjects were kept in the shock boxes for the same 20 min period without shock. The ambient temperature of the shock boxes did not exceed 0.4°C greater than the room temperature. The rats were then removed from the shock boxes, core temperature was immediately recorded, and the animals were then returned to the holding cages. Temperature measurements were repeated every 10 min for 90 min.

Experiment 2

Following the baseline temperature measurement each subject was pretreated with either an injection of 0.9% saline or naltrexone hydrochloride dissolved in distilled water (10.0 mg/kg; 1.0 ml/kg SC). After 15 min post-injection temperatures were recorded, and the rats moved into the shock boxes. The rats were randomly assigned to treatment groups and received either intermittent footshock of 0.5 or 2.5 mA current intensity or no shock for 20 min. The rats were then removed from the boxes and temperatures recorded as above.

Experiment 3

Subjects were rendered tolerant to morphine by implantation of morphine base pellets. On Day 1, a single morphine (75.0 mg) or placebo pellet was implanted SC behind the neck under halothane anesthesia. On Day 4 a second pellet was added and on Day 7 two pellets were added. On Day 10, baseline temperature was recorded and the rats were injected with 0.9% saline (1.0 ml/kg SC). After 15 min post-injection temperatures were recorded and each rat given in-

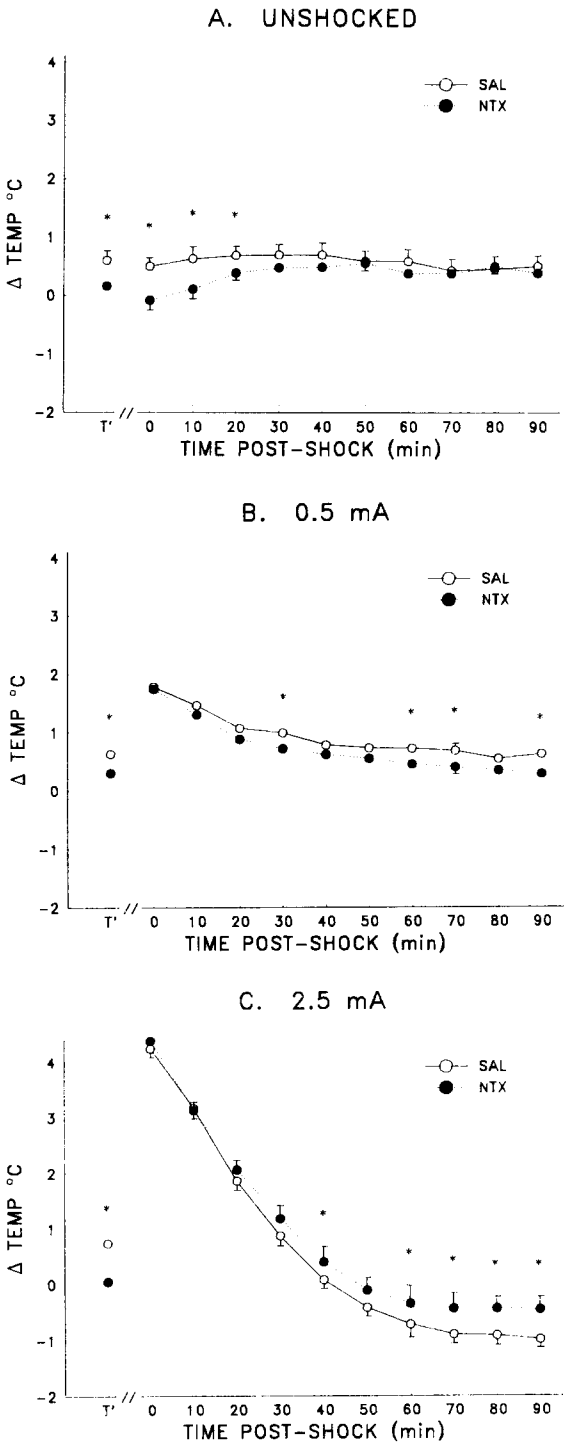

FIG. 2. The effects of saline or naltrexone pretreatment on the mean change in body temperature from baseline in unshocked subjects (A), and subjects receiving 20 min intermittent footshock of 0.5 mA (B) or 2.5 mA (C). T' is the change in temperature 15 min after the baseline temperature measurement and immediately prior to footshock. SAL=saline; NTX=naltrexone (10.0 mg/kg SC). Standard errors of the mean less than 0.1°C are not shown. N=8–11 per group. *$p<0.05$.

termittent footshock of either 0.5 or 2.5 mA current intensity or no shock for 20 min. Post-shock temperatures were recorded as described above.

Data Analysis

Temperature data were normalized by conversion into degrees change from the pre-injection baseline temperature. While the baseline values were normally distributed, the variances between groups were unequal following footshock. Therefore, statistical analysis was performed using nonparametric tests [24]. The sign test was used to test for significant changes in temperature between baseline and the measurement 15 min post handling and injection. In Experiments 2 and 3, Mann-Whitney U-tests were used to determine significant differences between groups at each time point. The level of significance was set at $p<0.05$ for all comparisons.

RESULTS

Experiment 1

There were no significant differences in baseline temperature between groups (38.3±0.07°C; mean±S.E.M., N=40); therefore, the groups were pooled in order to test for changes in temperature following the preliminary handling and saline injection. Handling produced a statistically significant increase of 0.37±0.05°C in core temperature (Fig. 1). Footshock was found to increase core temperature, with the degree of increase directly related to the current intensity. Extreme hyperthermia was noted after shock with the two highest currents (2.0 and 2.5 mA); some subjects having temperatures exceeding 42.0°C. Core temperature fell below baseline after footshock of 2.0 and 2.5 mA and remained low throughout the 90 min observation period. Temperatures of the unshocked controls had returned to baseline when tested immediately after removal from the shock boxes; however, their temperature increased again subsequent to repeated testing and remained elevated for the duration of the session.

Experiment 2

Pretreatment with naltrexone antagonized the initial handling-induced hyperthermia in all 3 groups (Fig. 2A, B and C). After removal of the unshocked rats from the shock boxes, the mean temperature of the naltrexone-pretreated group was significantly lower than that of the saline-pretreated group and tended to remain so for the duration of the observation; however, the temperature differences between these two groups diminished over time (Fig. 2A). This appeared to be due to a temperature increase over time in the naltrexone-pretreated rats rather than a temperature decrease in the saline-pretreated group. Naltrexone pretreatment had no effect on the peak temperature rise produced by footshock of either 0.5 or 2.5 mA (Fig. 2B and C). After footshock of 0.5 mA the temperature of naltrexone-pretreated subjects remained below the saline-pretreated subjects in a similar manner to the pattern observed in the unshocked groups. However, after footshock of 2.5 mA the temperature of naltrexone-pretreated subjects returned to baseline more slowly and also showed less rebound below baseline.

Experiment 3

There were no significant group differences in baseline temperatures between morphine and placebo-pelleted rats 10

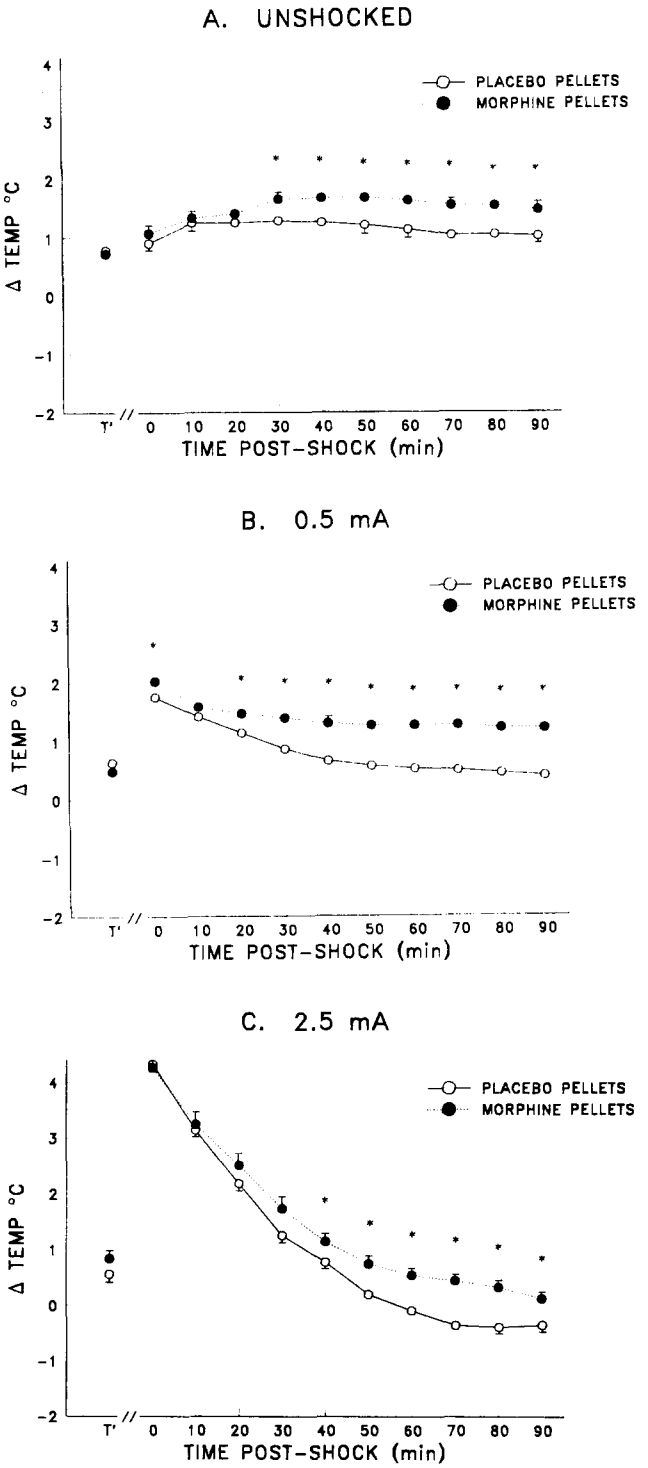

FIG. 3. The effects of chronic morphine or placebo pellet implantation on the mean change in body temperature from baseline in unshocked subjects (A), and subjects receiving 20 min intermittent footshock of 0.5 mA (B) or 2.5 mA (C). T′ is the change in temperature 15 min after the baseline temperature measurement and immediately prior to footshock. Standard errors of the mean less than 0.1°C are not shown. N=8–13 per group. *$p<0.05$.

days after the pelleting procedure was initiated (placebo, 38.11±0.07°C, N=32; morphine, 38.20±0.06°C, N=30), indicating that by this time tolerance had developed to the effects of morphine on body temperature. Chronic morphinization had no effect on handling-induced hyperthermia (Fig. 3A, B and C). After removal from the shock boxes and following repeated testing, the unshocked morphine-pelleted rats showed significantly higher core temperatures compared to unshocked placebo-pelleted controls (Fig. 3A). After footshock of 0.5 mA, the morphine tolerant rats showed a significantly greater rise in core temperature, and the temperature remained above the placebo-pelleted rats for the rest of the session (Fig. 3B). In contrast, chronic morphinization failed to affect the peak temperature rise following 2.5 mA of shock, and the temperature of the tolerant subjects did not return to baseline as quickly as placebo-pelleted rats nor did it show rebound hypothermia (Fig. 3C).

DISCUSSION

The results of the present study demonstrate that footshock can produce hyperthermia, the degree of which is a function of the intensity of the current delivered, i.e., the severity of the stressor. The peak increases in temperature following either low (0.5 mA) or high (2.5 mA) current footshock were not reduced by naltrexone pretreatment nor by chronic morphinization; in fact, chronic morphinization produced a slightly increased hyperthermic response to low current footshock (vide infra). These results indicate that the footshock-induced hyperthermia studied in this series of experiments was not mediated by endogenous opioid peptides. This conclusion is in conflict with the results of Millan *et al.* [19] who found that footshock-induced hyperthermia was antagonized by pretreatment with naloxone and decreased in chronically morphine-pelleted rats. A explanation for this discrepancy is that Millan *et al.* [19] used different shock parameters (3 mA, on 300 msec every 2 sec for 5 min) compared to those used in the present study (0.5 or 2.5 mA, on 1 sec every 5 sec for 20 min). It is conceivable that the longer period that the animals were exposed to footshock in the present study could have exhausted the supply of endogenous opioids, and a nonopioid factor was responsible for the observed hyperthermia. The extreme hyperthermia observed after 2.5 mA footshock was not due to increased locomotor activity as rats that are forced to run for 20 min only show a slight rise in core temperature of 1.3°C [25]. Thus, it appears that like footshock-induced analgesia [27], opioid and nonopioid forms of stress-induced hyperthermia occur. The substance or substances mediating the hyperthermia are not known. Preliminary tests revealed that pretreating the rats with indomethacin, propranolol or phentolamine did not affect the degree of hyperthermia (unpublished observations).

In contrast to the lack of involvement of opioids in the peak hyperthermia, opioids to appear to be involved in the return to baseline temperature following footshock at higher current intensities (2.5 mA). The return to baseline temperature was delayed in naltrexone-pretreated as well as in the chronically morphine-pelleted rats, and there was less rebound below baseline. Some opioids and opiates produce biphasic dose-response curves with respect to their effects on temperature; hyperthermia occurring at lower doses and hypothermia occurring at higher doses [6, 10, 18]. The severe stress produced by footshock and the resulting hyperthermia could have released large amounts of opioid peptides, and at high concentrations the hypothermic effects of these substances would have been predominant. An alternative explanation is that in response to the hyperthermia, opioids with hypothermic activity [9] are released as a compensatory mechanism. The finding that there was a rebound below baseline temperature in the saline-pretreated rats after footshock of 2.0 or 2.5 mA lends support to this hypothesis. These conclusions are in agreement with those of Holaday *et al.* [12] who have suggested that endogenous opioids are activated in response to heat stress and activate adaptive mechanisms. Holaday *et al.* [12] found that naloxone induced a rise in temperature in heat stressed rats accompanied by behavioral signs of hyperthermia and pointed out that "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". It is intriguing to consider that the analgesia observed after certain stressors may be secondary to opioid released due to activation of adaptive responses to stress-induced hyperthermia. In agreement with this supposition, Kulkarni [16] has found that heat exposure produces analgesia that is blocked by pretreatment with naloxone. Preliminary studies examining increases in body temperature induced by the SC injection of yeast indicated that such hyperthermia is not accompanied by analgesia as measured by the hot-plate test (unpublished observation), demonstrating that hyperthermia is not always associated with analgesia. The role of opioids in the return to baseline temperature after footshock of lower intensity (0.5 mA) is less clear. The pattern after footshock of 0.5 mA in the pelleted and the unpelleted groups was very similar to the handling effects observed in the unshocked controls (vide infra), suggesting that handling-induced hyperthermia was probably a large component of the temperature response.

In agreement with previous reports [2, 26, 29], the data indicate that handling-induced hyperthermia involves endogenous opioid peptides. In the present study this conclusion is based upon the blockade of handling-induced hyperthermia by prior treatment with naltrexone. However, the antagonism by naltrexone was short-lived and differences between saline and naltrexone pretreated-unshocked rats disappeared after several temperature measurements. This appears to be a reflection of a decrease of antagonism by naltrexone rather than habituation to the handling in the saline-pretreated rats as the temperature of the saline-pretreated rats remained fairly constant during the observation period. It is unlikely that the time dependence of this effect is related to the metabolism of naltrexone because the plasma half-life in the rat has been reported to be 1.4 hr [20]. A possible explanation for this phenomenon is that a second process is activated upon the initial stress, and after a latency period alters the stress-induced hyperthermia. In support of this hypothesis it has been reported that high doses of naloxone were ineffective in antagonizing stress-induced hyperthermia when given after the stress [19,26]. As stress, by definition, involves activation of the pituitary-adrenal axis, a conceivable mechanism could involve the release of adrenocorticotropic hormone or adrenal glucocorticoids. Adrenocorticotropic hormone as well as glucocorticoids have been reported to modify opiate-induced changes in temperature [8, 13–15], and concurrent activation of the pituitary-adrenal axis could alter the responsiveness to antagonism by naltrexone. In support of this conclusion, Ushijima *et al.* [28] have reported that stress can alter the effects of morphine on temperature and the ability of naloxone to reverse these effects.

No tolerance to the initial handling-induced hyperthermia was observed in chronically morphine-pelleted rats; in fact, the chronically morphinized rats revealed a greater degree of handling-induced hyperthermia compared to either placebo-pelleted or non-pelleted controls. However, the placebo-pelleted rats showed increased handling-induced hyperthermia compared to non-implanted rats, suggesting that the repeated surgery involved in implanting the pellets sensitized the rats to subsequent handling. Bläsig *et al.* [3] also found that chronically morphinized rats appear more disturbed during handling and show an exaggerated hyperthermic response to stress. In further agreement with the hypothesis that pelleting makes the animals more responsive to stress, Millan *et al.* [19] found elevated plasma β-endorphin levels in placebo-pelleted rats as compared to controls. However, as handling-induced hyperthermia is antagonized by naltrexone, one would also expect cross-tolerance with morphine if opioid peptides are involved. A possible explanation is that the exaggerated hyperthermia is due to an opioid peptide that acts through a non-mu receptor and therefore displays little cross-tolerance with morphine. The fact that handling-induced hyperthermia is antagonized by the large dose of naltrexone used in the present study does not preclude a non-mu receptor mechanism. In agreement with this hypothesis, Geller *et al.* [9] have described a class of opioids that produce only hyperthermia and require high doses of naloxone for blockade.

In conclusion, footshock can be used as a model system for inducing experimental hyperthermia. Endogenous opioid peptides appear to be involved in handling-induced hyperthermia, and while the increase in temperature after footshock does not involve opioids, the return to normal body temperature is facilitated by these endogenous substances. Many investigators have used identical footshock parameters, both acutely and chronically, in order to experimentally induce stress. The results of the present study indicate that the conclusions of such studies may be confounded by the production of severe, tissue damaging hyperthermia. Thus, studies using footshock as a stressor must be carried out with attention to the concurrent alterations in body temperature. The finding that temperature responses to stress are altered during chronic morphine administration may have importance with regard to thermoregulation in response to stress in patients receiving opiate analgesics.

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