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Evidence Against Involvement of β-Endorphin in Thermoregulation in the Cat

WESLEY G. CLARK, IOK-HOU PANG AND GARY L. BERNARDINI

Department of Pharmacology
The University of Texas Health Science Center at Dallas, Dallas, TX 75235

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CLARK, W. G., I.-H. PANG AND G. L. BERNARDINI. *Evidence against involvement of β-endorphin in thermoregulation in the cat.* PHARMACOL BIOCHEM BEHAV 18(5) 741–745, 1983.—In the cat naloxone has little, if any, effect on temperature under usual laboratory conditions and does not reduce febrile responses to leukocytic pyrogen. Hence, endogenous opioid peptides that are antagonized by naloxone are not essential for induction of fever or for maintenance of normal temperature in the absence of appreciable thermal stress. The purpose of this study was to assess the contribution of such endogenous opioids to thermoregulation in cats exposed to more severe thermal and non-thermal stresses. Changes in temperature of unanesthetized cats were determined after third cerebral ventricular injections of large doses (100, 250 μg) of naloxone or saline vehicle. Naloxone had no appreciable effect on the temperature of cats acutely exposed to hot (34°C) or cold (4°C) environments, either before or after tolerance to morphine had been induced by progressively greater daily or twice-daily intraventricular doses of 10–70 μg morphine sulfate. Naloxone also did not significantly affect the temperature of cats subjected to neck-restraint or forced to stand on a small platform if they were to avoid contact with ice water. These results provide no indication that an endogenous opioid peptide, such as β-endorphin, that is antagonized by naloxone contributes appreciably to thermoregulation in cats. They do not rule out the possibility that endogenous opioids, such as Met-enkephalin, that are not readily antagonized by naloxone are important for normal thermoregulation.

Cat Endogenous opioid peptides Morphine tolerance Naloxone Restraint Thermal stress Thermoregulation

SINCE the discovery of endogenous opioid peptides within the hypothalamus [27,29], a brain region of major importance to normal thermoregulation, numerous publications have described pharmacological effects of injected opioid peptides on body temperature [4, 10, 11, 20, 31]. Relatively low doses of β-endorphin increase temperature in the cat, mouse, rabbit and rat, and these responses have generally been inhibited by naloxone. In cats the stable enkephalin analogs D-Ala^2-Met-enkephalinamide [16], FK 33-824 [H_2N-Tyr-D-Ala-Gly-MePhe-Met(O)-OH] [12] and D-Ala^2-D-Leu^5-enkephalin (Pang, *et al.*, unpublished) also evoke hyperthermic responses that are reduced by naloxone. Although central administration of a parent peptide, Met-enkephalin, also evokes hyperthermia, it is not readily antagonized by naloxone [7,17] and is unlikely to act primarily at the receptor stimulated by β-endorphin [10]. Hence, naloxone should be more useful for assessing physiological role(s) of β-endorphin than of Met-enkephalin in thermoregulation in this species. Since central injections of β-endorphin increase body temperature, endogenous β-endorphin would be expected to do likewise, and naloxone would be expected to lower body temperature in situations in which β-endorphin is released for maintenance of temperature.

Naloxone and naltrexone usually have little or no effect on the temperature of animals housed at ordinary laboratory temperatures [13] so that in the absence of appreciable thermal stress any contribution of β-endorphin to thermoregulation must be minimal. Likewise, naloxone does not alter responses to pyrogens in cats [15] or rats [2] so that stimulation of naloxone-sensitive receptors is not involved in fever in these species.

The purpose of this study was to examine the additional possibilities that β-endorphin release contributes to thermoregulation in cats exposed to thermal or other types of stress. Naloxone was given centrally to non-tolerant and morphine-tolerant cats exposed to hot and cold environments and to cats restrained in a stock-like device or required to stand on a small platform for a prolonged period to avoid exposure to ice water. In none of these conditions was a marked change in body temperature associated with naloxone administration.

METHOD

Thirteen cats, 2.7–4.5 kg in weight, were used for this study. Except for tests involving heat or cold stress, they were kept in an environmental chamber at an ambient temperature of 22±1°C. For tests in the heat, the chamber temperature was increased to 34±1°C. For tests in the cold, the animals were moved to another chamber maintained at

4±2°C. Acute exposures to these extremes of environmental temperature began 60–90 min before an injection was given. Procedures for care and feeding of the animals, for automatically recording body temperature from the retroperitoneal space, for implantation of cannulas for injections into the third cerebral ventricle, for sterilizing glassware and avoiding pyrogenic contamination and for calculating thermal response indexes (TRIs) have been described previously [8,25]. TRIs are estimates of the area between a response curve and the base-line body temperature that was determined by averaging temperatures 10, 20 and 30 min before drug or vehicle injection. One TRI unit is equivalent to a 1°C change in temperature over a 1-hr period. Since the duration of action of the doses of naloxone (100, 250 μg) given in these experiments was estimated from previous studies [12,14] to be 3–4 hr, TRIs were calculated for the initial 3-hr period after injections. The paired *t*-test was used to evaluate differences between temperature changes after naloxone and vehicle administration [36]. The order in which each animal received naloxone and vehicle injections and/or was exposed to the different environments was determined with a table of random numbers. Prior to surgery, the cats received 2.2 mg/kg xylazine (Haver-Lockhart) SC. After they were sedated, 15–20 mg/kg pentobarbital sodium (Abbott) was injected IV.

Stock solutions of naloxone hydrochloride (NIDA) or morphine sulfate (Mallinckrodt) in 0.9% NaCl solution were stored at 4°C. Doses refer to these salts. Injections of these agents or saline vehicle into a ventricular cannula were in a volume of 0.05 ml. Residual naloxone was flushed from the cannula with 0.1 ml saline solution after completion of each daily experiment. Injections were given at 10:00 a.m. ±5 min.

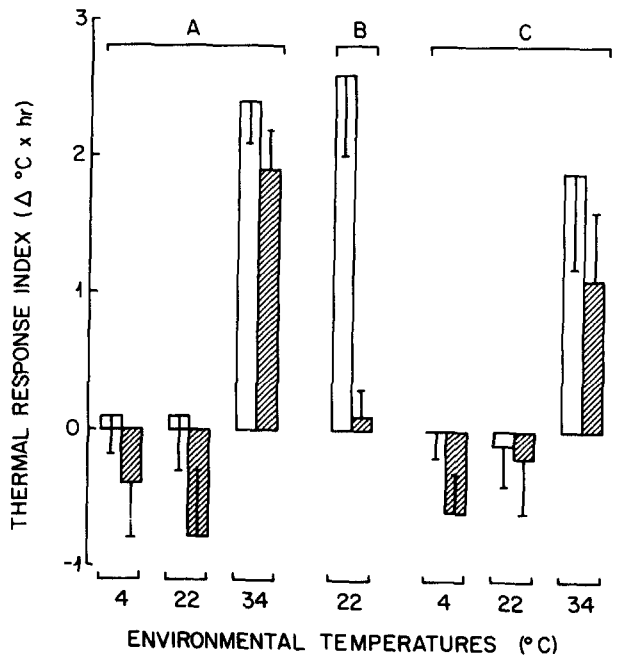

FIG. 1. Effect of ambient temperature on responses of six cats to third cerebroventricular injections of naloxone (250 μg). (A) Responses to saline vehicle (open bars) and naloxone (hatched bars) before induction of tolerance to morphine. (B) Response of the same cats to an initial intraventricular dose of 10 μg morphine (open bar) and lack of response to 70 μg morphine after development of tolerance (hatched bar). (C) Responses of the same animals to naloxone and saline after development of tolerance to morphine, illustrated as in panel A. Mean values are indicated ±SEM.

RESULTS

Effect of Naloxone on the Temperature of Non-Tolerant Cats Exposed to Various Ambient Temperatures

Cats were given a series of injections of naloxone or vehicle at each of three ambient temperatures. Although there was a reduction in mean body temperature after naloxone in all three environments (Fig. 1A), this was always slight and reached statistical significance ($p<0.05$) only in the hot environment. In the heat the temperature of cats gradually increases without treatment or after vehicle administration [14], accounting for the relatively large and positive control TRI. The mean difference between TRIs of 0.5°C×hr over the 3 hr in the heat was equivalent to a difference between the two treatments of less than 0.2°C throughout this period.

Effect of Naloxone on the Temperature of Morphine-Tolerant Cats Exposed to Various Ambient Temperatures

Following the above tests, the same cats were given a dose of 10 μg morphine, which evoked typical hyperthermic responses (Fig. 1B), and they also were tested with a hyperthermic dose of the synthetic opioid peptide, D-Ala²-D-Leu⁵-enkephalin. Over the next 4–11 weeks they received twice daily or daily injections of morphine. The dose was increased in 10 μg increments as tolerance developed until no appreciable response was elicited by administration of 70 μg morphine sulfate (Fig. 1B). This required 35–85 morphine injections. It can be estimated from a previous determination of the dose-response relationship [14] that, at this level of tolerance, at least a fourfold increase in morphine dosage, i.e., 280 μg or 28 times the initial dose, would have been necessary to elicit a response comparable to the initial response. At this time D-Ala²-D-Leu⁵-enkephalin still elicited hyperthermias comparable to those before tolerance to morphine (Pang, *et al.*, unpublished data) so that the animals were still able to generate increases in temperature to other opioid stimuli. The animals were then retested with naloxone and vehicle over the same range of ambient temperatures, and again no significant changes in body temperature were induced by the antagonist (Fig. 1C). Vocalization, which usually lasted no more than 10 min after naloxone injection, was the only consistent behavioral change noted, although some of the animals initially appeared somewhat agitated as well. Tolerance was maintained during this period by continued administration of 70 μg doses of morphine between vehicle and naloxone tests.

Effect of Naloxone on the Temperature of Cats Subjected to Neck-Restraint

On two occasions, separated by 1 week to minimize habituation to the situation, cats that had never previously been subjected to restraint were fastened around the neck in a stock-like device [33]. Naloxone (100 μg) or vehicle was injected approximately 20 min before placing an animal in the device, with the order of the two tests assigned randomly so that five cats received naloxone in the initial test and four received vehicle. Three of the animals, two given naloxone and one given saline, initially vocalized, struggled vigorously and attempted to escape after being placed in the device for

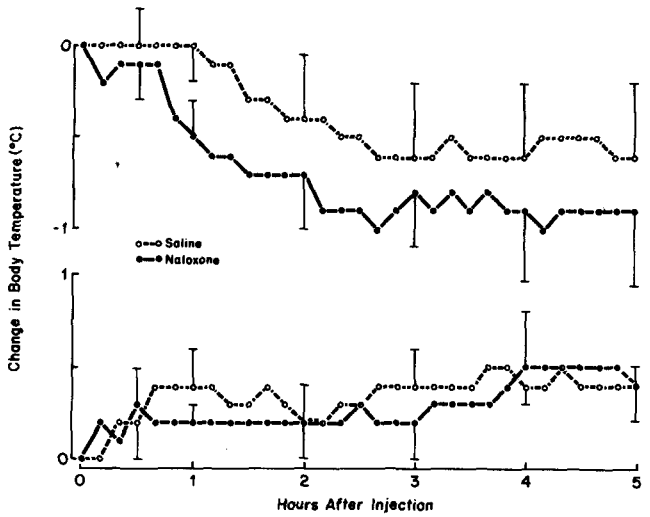

FIG. 2. Effect of naloxone on body temperature of cats subjected to physical stress. Top: Nine cats were restrained about the neck beginning 10–30 min after third ventricular injection of naloxone (100 μg) or saline vehicle. Bottom: Cats (N=6) were placed on a small platform surrounded by ice water beginning 1–15 min after injection of naloxone (100 μg) or vehicle. Mean values are shown ±SEM.

the first time, and body temperature increased 0.6–1.0°C during the initial 10–30 min period. Then they settled down and, for the most part, rested until the end of the session. The other animals displayed less agitation, usually with intermittent vocalization and an increase in respiratory rate of 10–40 breaths/min. The second test caused less disturbance than the first regardless of the pretreatment. Naloxone did not significantly alter body temperature (Fig. 2, top). Two animals exhibited hypothermias of 2.2 and 4.0°C, respectively, after naloxone and 3.2 and 2.7°C after vehicle. Both received naloxone in their initial trial. These two cats account for much of the reduction in mean body temperatures. The temperatures of the other seven animals changed relatively little after vehicle administration but did tend to decrease somewhat after naloxone.

Effect of Naloxone on the Temperature of Cats Forced to Stand to Avoid Contact with Ice Water

Cats were placed on a small platform (7.5×7.5 in.) consisting of two bricks surrounded by water on the floor of the cage. The water was kept cold by the addition of ice. Naloxone or vehicle was injected approximately 10 min before beginning the test. The idea was that to avoid contact with the water the cats would have to stand carefully on the platform, which was too small to lie down on, and that eventually this situation would be stressful and perhaps unmask an endogenous opioid contribution to temperature control. At first the animals vocalized and appeared quite distressed. They often stepped momentarily into the water and then back onto the platform. Some leaned on the front of the cage with their forepaws while keeping their hindpaws on the platform. Within an hour they all chose to sit in the water. Although the situation would seem to be stressful to cats whether they stayed on the platform or not, body temperatures in the control tests changed very little (Fig. 2, bottom), and naloxone had no effect on body temperature in this situation.

DISCUSSION

The lack of appreciable effect of naloxone on body temperature of normal cats exposed to heat or cold is in general agreement with results in other species [13] and reinforces previous evidence that β-endorphin is unlikely to contribute significantly to normal thermoregulation. The temperature of rats [18, 22, 38] or mice [4,32] exposed to heat did not significantly change, decreased or increased slightly after administration of naloxone or naltrexone. In a cold environment in one study [38] naloxone and naltrexone lowered the temperature of rats 0.4–0.8°C, but in other studies naloxone had even less effect [2, 13, 28, 39, 40]. Naloxone given peripherally to mice [4] or into the preoptic region of rabbits [31] did not change their temperature in the cold. It should be noted that the dose of naloxone used in this phase of the present study was 2.5 times a dose that abolished hyperthermic responses to injected β-endorphin [11] so it is unlikely that a larger dose would have been more effective.

When animals that respond to acute administration of morphine with hyperthermia are made tolerant to morphine or other opioids, they often respond to naloxone administration with a withdrawal hypothermia [13], and there is evidence of a similar response in rats pretreated with a single dose of β-endorphin [24]. After French *et al.* [21] had induced tolerance in cats to the hyperthermic effect of morphine by a series of 12 daily IV doses, injection of naloxone by the same route evoked hypothermic responses that were greater in the animals made tolerant to larger doses of morphine. Their animals had developed less than fourfold tolerance after six prior opiate injections, but the level of tolerance at the end of the full series of injections was not reported. We tested the effect of naloxone at three environmental temperatures in morphine-tolerant animals to assess whether the expected withdrawal hypothermia was caused by a lowering of the level at which body temperature was regulated, as suggested for rats [1] or to a less specific disruption of thermoregulation. If the former mechanism were correct, body temperature should have decreased after naloxone in all three thermal environments; if the latter were true, body temperature would still decrease in the two cooler environments but it should have increased in the heat [9]. However, in contrast to the results of French *et al.* [21], naloxone had no appreciable effect, even under conditions of thermal stress, in our animals that were probably more tolerant. It is unlikely that the difference in routes can account for the discrepancy between these two studies since morphine-induced temperature changes, tolerance and dependence are centrally mediated phenomena regardless of the route of administration. Though our animals were markedly tolerant to morphine, they gave no indication of dependence involving the thermoregulatory system. Schulz *et al.* [34,35] have also recently reported a dissociation between tolerance and physical dependence on opioids in the mouse vas deferens that was markedly tolerant to D-Ala²-D-Leu⁵-enkephalin or sufentanyl.

Studies with naloxone and naltrexone have indicated that endogenous opioid release can increase the temperature of rats exposed to non-thermal types of stress and/or that stress may mask a contribution to normal thermoregulation in this species [2, 3, 19, 37, 38]. We were unable to obtain support for such a role in the cat. The temperature of neither vehicle- nor naloxone-pretreated, restrained cats changed consistently; increasing transiently in some that struggled, not changing or decreasing in others. Intraventricular doses

of 20–100 μg naloxone effectively reduce hyperthermic responses of cats to opioids such as morphine [8], β-endorphin [11], D-Ala²-Met-enkephalinamide [16] and FK 33-824 [12] for at least 2 hr so that the lack of significant differences between vehicle and naloxone tests cannot be attributed to an insufficient duration of naloxone action. In view of the usual aversion cats have to water, the ice water should have been stressful. There were behavioral indications that such was the case, but there was no appreciable shift in temperature relative to saline controls after naloxone was given.

Thus the present studies provide no evidence that β-endorphin has a role in thermoregulation in the cat, and we are left with an unsatisfying paradox. A variety of peptide and non-peptide opioids, i.e., β-endorphin, D-Ala²-Met-enkephalinamide, FK 33-824, D-Ala²-D-Leu⁵-enkephalin and morphine, have quite effectively altered thermoregulation in this species in our previous studies, and each was antagonized by central injection of 5–25 μg doses of naloxone. So it is clear that these agonists have potent pharmacological effects on thermoregulation, although they apparently act on a variety of naloxone-sensitive receptors [10]. Yet we have been unable to obtain data that support a contribution of opioids acting via naloxone-sensitive receptors to physiological control of temperature in the cat. It may be relevant that although β-endorphin immunoreactivity has been found in fibers in the preoptic/anterior hypothalamus [5,6], a region that is intimately involved in thermoregulation, cell bodies within the central nervous system that exhibit β-endorphin immunoreactivity have been located only in the basal hypothalamus [5, 6, 26, 29], primarily in the arcuate nucleus, a region that has not yet been documented to be of importance to thermoregulation.

The lack of response to naloxone does not mean that all endogenous opioid peptides are unimportant for normal thermoregulation. Met-enkephalin immunoreactivity has been found in both cell bodies and fibers within the preoptic/anterior hypothalamus [23,29]. The possibility still exists that endogenous peptides, such as Met-enkephalin and dynorphin [30], that are poorly antagonized by naloxone have important roles in thermoregulation.

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