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BRIEF COMMUNICATION

# Hyperthermia in the Rat From Handling Stress Blocked by Naltrexone Injected Into the Preoptic-Anterior Hypothalamus

YOUNG-SOOK PAE,[1] HENRY LAI[2] AND AKIRA HORITA

*Departments of Pharmacology, Psychiatry and Behavioral Sciences, University of Washington, School of Medicine, Seattle, WA 98195*

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PAE, Y.-S., H. LAI AND A. HORITA. *Hyperthermia in the rat from handling stress blocked by naltrexone injected into the preoptic-anterior hypothalamus.* PHARMACOL BIOCHEM BEHAV 22(2) 337–339, 1985.—Experimental handling and colonic temperature measurement have been shown to cause stress and induce a long-lasting rise in colonic temperature in the rat. This stress-induced hyperthermia was blocked by microinjection of the narcotic antagonist naltrexone into the preoptic-anterior hypothalamus (POAH) of the brain, but was not significantly affected by similar injections into areas of the brain above the POAH. Thus, the stress-induced hyperthermia may be caused by activation of the endogenous opioid mechanism in the POAH.

Handling stress Hyperthermia Naltrexone Preoptic-anterior hypothalamus

STRESS induces changes in body temperature in a variety of animal species, including man [1, 3, 5, 13]. In the rat, handling and temperature measurement by rectal probe result in a long-lasting rise in body temperature [3,12]. The hyperthermia is blockable by the narcotic antagonist naloxone, injected intraperitoneally immediately before handling [14]. The hyperthermia is also attenuated by intracerebroventricular injection of (−)-naloxone, but is unaffected by the inactive isomer (+)-naloxone [2]. These data suggest the involvement of central endogenous opioids in the hyperthermia.

Since the preoptic-anterior hypothalamus region (POAH) of the brain is involved in thermoregulation and injection of endogenous opioids into the POAH had been shown to induce hyperthermia in the rat [7], we decided to investigate the effects of injection of the specific narcotic antagonist naltrexone into the POAH on hyperthermia induced by handling stress in the rat.

## METHOD

### *Animals*

Male Sprague-Dawley rats (250–300 g), obtained from Tyler Laboratories, Bellevue, WA, were used in these experiments. They were housed four to a cage in a temperature-controlled vivarium maintained on a 12-hr light-dark cycle (light cycle between 8:00 and 20:00 hr). They were provided with food and water ad lib.

### *Intracerebral Injection*

The rats were anesthetized with intraperitoneal injection of 50 mg/kg of sodium pentobarbital and given 1.0 mg/kg of atropine methyl bromide. A guide cannula (23 g, 15 mm in length) was implanted unilaterally in the brain by stereotaxic technique with the tip positioned 3.0 mm above the site of injection. At least 5 days after the surgery, 5 μg of naltrexone (Dupont Pharmaceutical, Garden City, NY; dissolved in 1 μl of sterile, pyrogen-free 0.9% saline) or 1 μl of 0.9% saline was injected into the brain with a 30 g injection cannula (18 mm) inserted through the guide cannula. With the rat manually restrained, intracerebral injection was started 30 sec after the insertion of the injection cannula at a rate of 1 μl/min. The injection cannula was left in the brain for an additional 30 sec after injection before withdrawal. To minimize contamination with pyrogen, we used aseptic needles, syringes, and drugs. The coordinations of intracerebral injection were derived from the rat brain atlas of Paxinos and Watson [11]: they were in the general area of the preoptic-anterior hypothalamic region, AP, +7.7 mm; L, 0.7 mm; and DV, +1.2 mm; and the septal region, AP, +8.7 mm; L, 0.8 mm; and DV, +4.0 mm. Sites of injection were verified at

[1]Permanent address: Department of Pharmacology, Ewha Woman's University, School of Medicine, Seoul, Korea.

[2]Requests for reprints should be addressed to Henry Lai, Ph.D., Department of Pharmacology, SJ-30, University of Washington, Seattle, WA 98195.

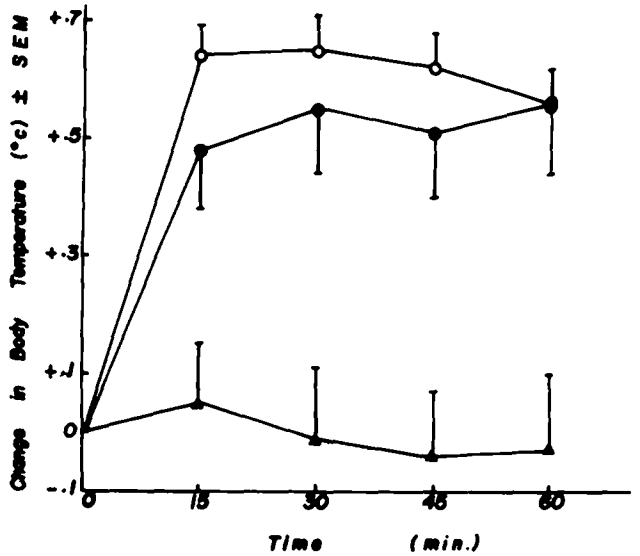


FIG. 1. Changes in colonic body temperature in rats injected intracerebrally (at time zero) with naltrexone (▲) (n=8) or saline (●) (n=13), and in rats subjected to the handling procedure without intracerebral injection (○) (n=9). The mean (±SEM) body temperature of the rats at time zero was 38.1±1°C.

the end of the experiments. One microliter of India ink was injected to the brain site with the injection cannula. The rats were then sacrificed by decapitation, their brains were dissected out and cut into coronal sections, and these were examined for the exact sites of injection.

*Experimental Design and Procedure*

All experiments were performed between 11:00 and 13:00 hr at an ambient temperature of 22°C. The rats were moved from the vivarium to the laboratory at least two hours before the experiment. This allowed enough time for them to habituate to the experimental environment and to recover from the disturbance of transportation. They were then given the intracerebral injection. Colonic temperature was monitored immediately before and at 15, 30, 45 and 60 min after the injection with a YSI-402 thermister probe inserted 8 cm into the rectum, and temperature was recorded by a YSI-43TG telethermometer (Yellow Springs Instruments, Yellow Springs, OH). During temperature measurements, the rats were manually restrained lightly by the base of the tail. Each temperature measurement took approximately 30 sec, after which the probe was withdrawn. The animals were kept (four to a cage) in their home cages between temperature measurements.

In a separate experiment, the effect of handling and temperature measurement without the intracerebral injection was studied in experimentally naive rats. Body temperature in these rats was monitored at 15-min intervals for 60 min as described above, but without the intracerebral injection. These animals had also unilateral intracerebral cannula implantation for control of the possible effect of surgery.

Each animal was used only once in the experiments.

*Data Analysis*

Data were plotted as change in colonic temperature from the first temperature measurement versus time. Temperature response curves were compared by the nonparametric statistical method of Krauth [6]. Curves were fitted into or-

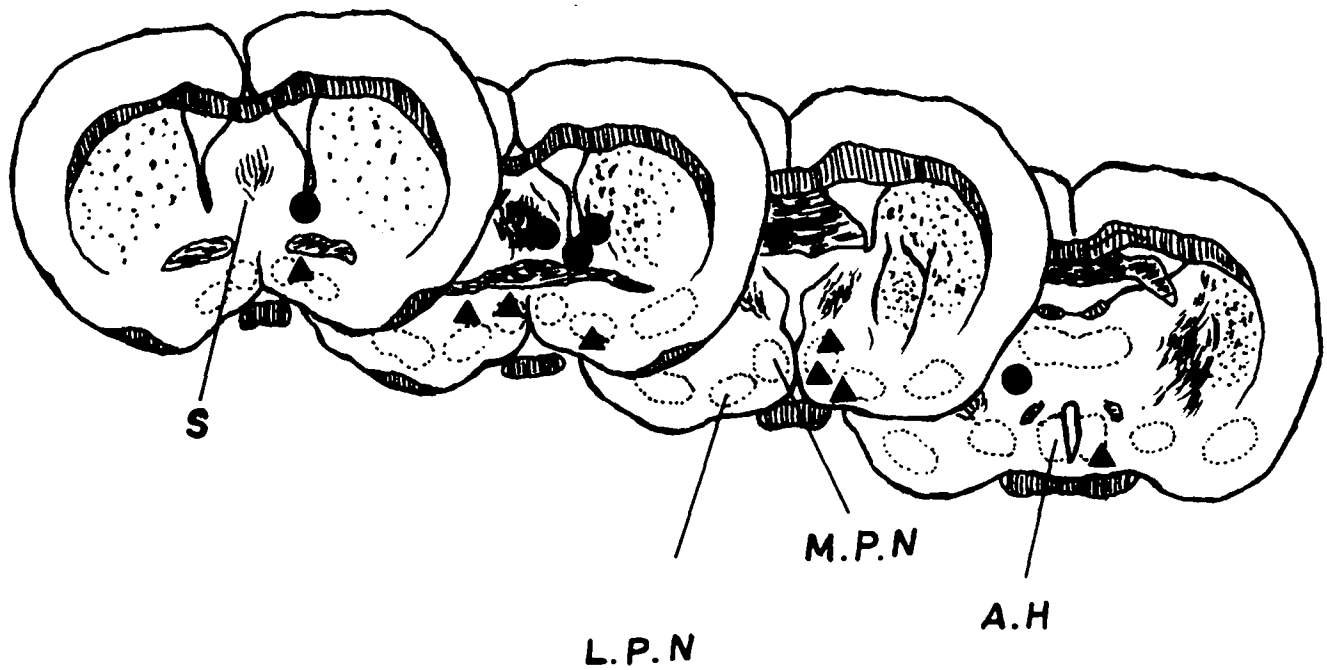


FIG. 2. Coronal sections of the rat brain showing sites of intracerebral naltrexone injection. A.H., anterior hypothalamus; L.P.N., lateral preoptic nucleus; M.P.N., medial preoptic nucleus; S, septum.

thogonal polynomials, and the zero-order polynomial coefficients were compared by the two-tailed Mann-Whitney U-test. Difference with $p<0.05$ was considered statistically significant.

## RESULTS AND DISCUSSION

Changes in colonic temperature in the rats as a function of time are shown in Fig. 1. Increase in colonic temperature was observed in rats that received intracerebral (both in the POAH and brain areas above the POAH) saline injections and also in rats subjected to the handling and temperature measurements without the intracerebral injection. However, there was no significant difference between the hyperthermic responses of these two treatment groups. Thus, the additional intracerebral injection procedure did not further contribute to the hyperthermia from handling stress.

The hyperthermia was completely blocked in rats that received naltrexone injection in the POAH (filled triangles in Fig. 2). In these animals, colonic temperature was virtually unchanged during the hour when temperature was measured. (Responses of intracerebral-saline-injected vs. POAH-naltrexone-injected rats, $U=90.5(8,13)$, $p<0.005$; and responses of handling-only vs. POAH-naltrexone injected rats, $U=71(8,9)$, $p<0.001$.) However, rats that received naltrexone injection into the brain regions above the POAH (filled circles in Fig. 2) developed hyperthermia not significantly different from that of the intracerebral-saline-injected animals. The brain areas injected included the lateral septum and the medial part of the neostriatum. The changes in colonic temperature of these animals (°C±SEM, n=5) at 15, 30, 45 and 60 min after intracerebral injection were 0.49±0.24, 0.37±0.24, 0.44±0.23, and 0.42±0.23, respectively.

The fact that the hyperthermia induced in the rats by handling was blocked by intracerebral injection of the specific narcotic antagonist naltrexone suggests the involvement of central endogenous opioids. Furthermore, the POAH was shown by our experiments to be a major region of the brain involved in the stress-induced hyperthermia, since the hyperthermia was completely blocked by injection of naltrexone into that region. Site specificity is confirmed by the ineffectiveness of naltrexone to block the hyperthermia when injected into areas of the brain above the POAH.

Ample evidence has implicated the involvement of endogenous opioids in the hyperthermia from handling stress [2,14] and activation of the opioid-related thermoregulatory mechanism by stress. For example, the thermal effect of morphine has been shown to be potentiated by stress from such experimental factors as restraint [8,9] and handling [15].

Stress has been shown to activate both central and peripheral β-endorphins in the rat, and such release has been related to the hyperthermia [10]. Pituitary β-endorphin seems to play an important role in the hyperthermia from handling stress since the hyperthermia can be attenuated by hypophysectomy [14] and treatment with dexamethasone [4]. It is likely that the released pituitary β-endorphin reenters the brain and acts on opioid receptors in the POAH to produce the hyperthermia, although the involvement of central β-endorphin and other endogenous opioids cannot be ruled out.

## ACKNOWLEDGEMENT

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## REFERENCES

1. Bartlett, R. G., Jr., V. C. Bohr, R. H. Helmendach, G. L. Foster and M. A. Miller. Evidence of an emotional factor in hypothermia produced by restrain. *Am J Physiol* **179:** 343–346, 1954.
2. Bläsig, J., V. Hölt, U. Bäuerle and A. Herz. Involvement of endorphins in emotional hyperthermia of rat. *Life Sci* **23:** 2525–2532, 1978.
3. Briese, E. and M. G. de Quijada. Colonic temperature of rats during handling. *Acta Physiol Lat Am* **20:** 97–102, 1970.
4. French, E. D. Dexamethasone blocks morphine-induced hypothermia in restrained rats. *Life Sci* **25:** 1583–1590, 1979.
5. Grant, R. Emotional hypothermia in the rabbits. *Am J Physiol* **160:** 285–290, 1950.
6. Krauth, J. Nonparametric analysis of response curves. *J Neurosci Methods* **2:** 239–252, 1980.
7. Martin, G. E. and C. B. Bacino. Action of intracerebrally injected β-endorphin on the rat's core temperature. *Eur J Pharmacol* **59:** 227–236, 1979.
8. Martin, G. E. and J. E. Morrison. Hyperthermia evoked by the intracerebral injection of morphine sulfate in the rat: the effect of restrain. *Brain Res* **145:** 127–140, 1978.
9. Martin, G. E. and N. L. Papp. Effect on core temperature of restraint after peripherally and centrally injected morphine in the Sprague-Dawley rat. *Pharmacol Biochem Behav* **10:** 313–315, 1979.
10. Millan, M. J., R. Przewlocki, M. Jerlicz, C. H. Gramsch, V. Höllt and A. Herz. Stress-induced release of brain and pituitary β-endorphin: major role of endorphins in generation of hyperthermia, not analgesia. *Brain Res* **208:** 325–338, 1981.
11. Paxinos, G. and C. Watson. *The Rat Brain in Stereotaxic Coordinates*. New York: Academic Press, 1982.
12. Poole, S. and J. D. Stephenson. Core temperature: some shortcomings of rectal temperature measurements. *Physiol Behav* **18:** 203–205, 1977.
13. Renbourn, E. T. Body temperature and pulse rate in boys and young men prior to sporting contests: a study of emotional hyperthermia with a review of the literature. *J Psychosom Res* **4:** 149–175, 1960.
14. Stewart, J. and R. Eikelboom. Stress masks the hypothermic effect of naloxone in rats. *Life Sci* **25:** 1165–1172, 1979.
15. Stewart, J. and R. Eikelboom. Interaction between the effects of stress and morphine on body temperature in rats. *Life Sci* **28:** 1041–1045, 1981.