Pharmacology Biochemistry & Behavior, Vol. 23, pp. 505–507, 1985. 0091-3057/85 $3.00 + .00

Intraseptal Morphine Potentiates Pentobarbital Narcosis and Hypothermia in the Rat

SAHBA FATHERAZI, HENRY LAI,[1] SALAHUDDIN KAZI AND AKIRA HORITA

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

Received 14 December 1984

FATHERAZI, S., H. LAI, S. KAZI AND A. HORITA. *Intraseptal morphine potentiates pentobarbital narcosis and hypothermia in the rat.* PHARMACOL BIOCHEM BEHAV **23**(4) 505–507, 1985.—Morphine injected intraseptally in the amounts of 35 and 70 nmol prolonged pentobarbital-induced narcosis in the rat. Pentobarbital-induced hypothermia was also potentiated by intraseptal injection of 70 nmol of morphine. These effects were antagonized when morphine was injected together with naltrexone (29 nmol). Naltrexone injected by itself into the septum did not significantly affect pentobarbital-narcosis and hypothermia. It is concluded that activation of mu opioid receptors in the septal region could affect the actions of pentobarbital.

Morphine Naltrexone Pentobarbital Narcosis Body temperature Septum

MODULATION of the septal functions by a variety of afferents from different regions of the brain has been shown by many studies [5, 6, 8, 9]. For example, activity of the septohippocampal cholinergic pathway, a major septal efferent, is modified by dopaminergic, adrenergic, endogenous opioids and glutaminergic inputs to the septum. Other studies have shown that activity of the septohippocampal pathway affects the biochemical [1] and behavioral [2] effects of pentobarbital. In particular, activation of this pathway antagonizes the narcotic effect of pentobarbital, as evidenced by a shortening of the duration of loss of righting reflex in animals injected with the drug. However, the effect of decreased septohippocampal cholinergic activity on the action of pentobarbital has thus far not been investigated. The present study concerns the effect of intraseptal morphine on pentobarbital-induced narcosis and hypothermia. Morphine injected intraseptally has been shown to decrease hippocampal cholinergic activity [10].

METHOD

Animals

The experiments were performed on male Sprague-Dawley rats (250–300 g) purchased from Tyler Laboratories, Bellevue, WA. They were housed in a vivarium maintained on a 12-hr light-dark cycle (light on 8 a.m.-8 p.m.) and provided with food and water ad lib.

Procedure

At least 5 days prior to the behavioral experiment, the animals were anesthetized with pentobarbital (25 mg/kg, IP) and given 1 mg/kg atropine bromide. A 23 gauge guide cannula was implanted stereotaxically in the brain, its tip positioned 3.0 mm above the septal area. Coordinates of the septal injection site were AP+9.7 mm, DV+5.0 mm and L ± 0.3 mm with reference to the interaural line in accordance with the rat brain stereotaxic atlas of Paxinos and Watson [6]. The animals were moved to the research laboratory 12 hr before the behavioral experiment for habituation. At the start of the experiment the rats were weighed and allowed to recover for 30 min from the handling effect. A challenge dose of sodium pentobarbital (50 mg/kg, IP) was then given at time zero. Body temperature was measured every 15 min for 180 min from zero time by a temperature probe (YSI-402 thermister, Yellow Springs Instrument) inserted 8 cm via the rectum. The ambient temperature during the experiment was 22°C. At 30 min after zero time, a 30 gauge injection cannula was inserted into the guide cannula to a point 3.0 mm beyond its tip. Thirty seconds later 1 μl of pyrogen-free physiological saline or 1 μl of the drug dissolved in physiological saline was injected. The rate of injection was 1 μl/min. Different groups of animals were injected intraseptally with 17.5, 35 and 70 nmol of morphine, or morphine with 29 nmol of naltrexone. The injection cannula was withdrawn 30 sec after completion of the injection. The animals were then placed on their backs and the time of return of righting reflex was recorded.

In a separate group of rats, the effect of intraseptal morphine administration alone on body temperature was studied. Seventy nmol of morphine was injected intraseptally into the conscious rats at time zero and changes in body tem-

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

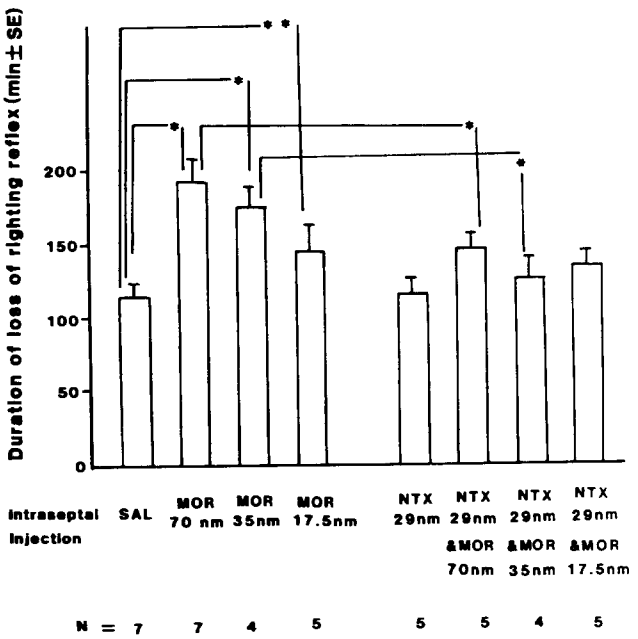

FIG. 1. Effects of intraseptal microinjection of different concentrations of morphine and naltrexone on pentobarbital-induced narcosis. nm, amounts of morphine and naltrexone injected in nmol; *$p<0.01$; **no significant difference; SAL, saline; MOR, morphine; NTX, naltrexone.

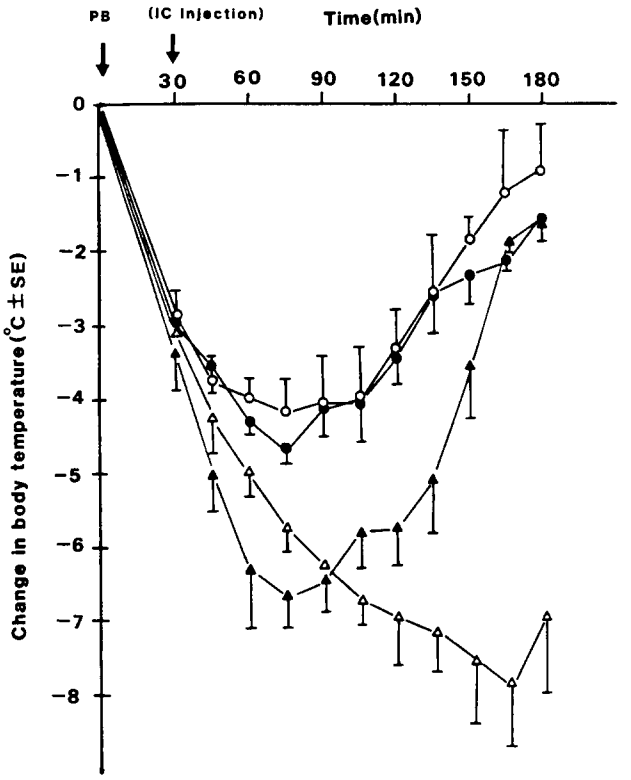

FIG. 2. Effects of intraseptal injection (IC) of morphine (70 nmol), naltrexone (29 nmol), and morphine (70 nmol) + naltrexone (29 nmol) on pentobarbital-induced hypothermia. Control animals were injected intraseptally with 1 μl saline. ○——○, saline; △——△, morphine (70 nmol); ●——●, naltrexone (29 nmol); ▲——▲, morphine (70 nmol) + naltrexone (29 nmol). Each curve represents the results from at least five rats. Pentobarbital was injected (IP) at time zero and morphine and naltrexone injections were given 30 min later.

perature were monitored as described above. Control animals received intraseptal injections of 1 μl of pyrogen-free physiological saline.

At the end of the experiments, 1 μl of India ink was injected intraseptally with the injection cannula, the animals were sacrificed by decapitation and the location of the injection site was confirmed by histologic inspection.

Data Analysis

Data from the narcosis study were analyzed by the one-way analysis of variance, and the differences between groups were compared by the Newman-Keul's test. Temperature response curves were compared by the nonparametric statistical method of Krauth [3]. A difference at $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The data on the effect of morphine on pentobarbital-induced narcosis are presented in Fig. 1. Morphine in the amounts of 70 and 35 nmol potentiated the narcotic effect of pentobarbital ($p<0.01$ compared with controls), but at 17.5 nmol had no significant effect. The effects of morphine (70, 35 nmol) were completely antagonized when it was injected together with naltrexone. However, naltrexone injected by itself did not significantly affect the narcosis.

The effects of intraseptal injection of 70 nmol of morphine on pentobarbital-induced hypothermia are presented in Fig. 2. The data are plotted as change in body temperature from that at zero time. Morphine significantly enhanced the hypothermic effect of pentobarbital ($p<0.002$). Naltrexone injected by itself did not have any significant effect on pen-

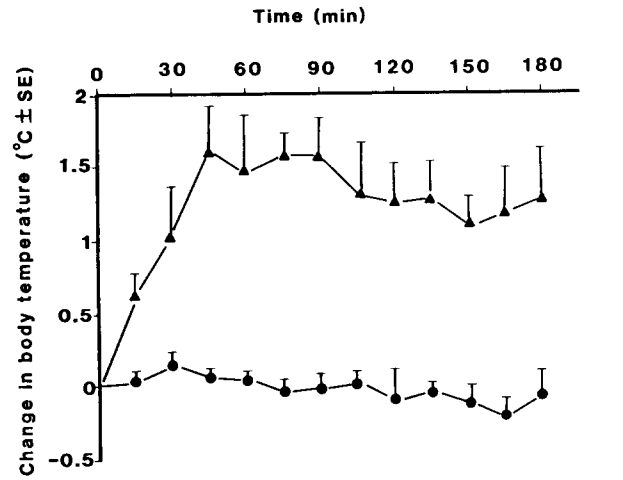

FIG. 3. Effects of intraseptal injection of morphine (70 nmol) (▲) on body temperature of the conscious rats. Control animals (●) were injected intraseptally with 1 μl of saline. Intraseptal injection was given at time zero. Each curve represents the results from six rats.

tobarbital-induced hypothermia, but in combination with morphine, it significantly attenuated though it did not completely antagonize the effect of morphine on the hypothermia. Hypothermic responses of the "morphine + naltrexone"-injected rats are significantly less than the morphine-injected rats ($p<0.05$). However, the temperature responses of the "morphine + naltrexone"-injected rats are significantly more hypothermic than those of the "saline"-injected controls ($p<0.05$).

The effects of intraseptal injection of 70 nmol of morphine on the body temperature of the conscious rats are presented in Fig. 3. Morphine injected into the septum induced a hyperthermic response in the rat ($p<0.005$, comparing responses of morphine-injected rats vs responses of saline-injected controls).

It is thus apparent that intraseptal injection of morphine potentiates pentobarbital-induced narcosis in a dose-dependent manner in the rat. The fact that the effect of morphine was abolished by concomitant intraseptal naltrexone administration suggests that morphine exerts its effects through mu opioid receptors. Morphine has been proposed to stimulate mu opioid receptors and naltrexone is a potent antagonist at these receptors [4]. It may also be speculated that morphine potentiates pentobarbital narcosis by decreasing the activity of the septohippocampal cholinergic system. Activity in that system has been shown to affect arousal from pentobarbital narcosis [1,2], and the dosage of morphine injected in our experiment (70 nmol) has been shown to decrease the turnover rate of acetylcholine in the hippocampus [11]. It is of interest to note that whereas naltrexone completely blocked the effect of morphine on the narcosis, it was unable to block the hypothermic effect of pentobarbital. The maximal fall in temperature of the naltrexone-and-morphine-treated animals was similar to that of the morphine-treated rats, but the recovery was at a faster rate. In addition, it is also surprising to find that intraseptal morphine potentiated the hypothermia in pentobarbital-treated rats but caused a hyperthermic response in conscious animals. These results suggest a complex action of intraseptal morphine on body temperature and mediation of its effects on pentobarbital narcosis and hypothermia by independent neuronal mechanisms. Furthermore, even though morphine injection was made into the septum, its actions on other brain sites due to diffusion cannot be excluded.

ACKNOWLEDGEMENTS

Dr. Sahba Fatherazi was supported by a grant from the American Association of University Women (AAUW). This research was partially supported by a grant from the Alcoholism and Drug Abuse Institute of the University of Washington.

REFERENCES

1. Brunello, N. and D. L. Cheney. The septal-hippocampal cholinergic pathway: Role in antagonism of pentobarbital anesthesia and regulation by various afferents. *J Pharmacol Exp Ther* **219:** 489-495, 1982.
2. Kalivas, P. W. and A. Horita. Involvement of the septohippocampal system in TRH antagonism of pentobarbital narcosis. In: *Thyrotropin-Releasing Hormone*, edited by E. C. Griffiths and G. W. Bennett. New York: Raven Press, 1983, pp. 283-289.
3. Krauth, S. Nonparametric analysis of response curves. *J Neurosci Methods* **2:** 239-252, 1980.
4. Martin, W. R., C. G. Eades, J. A. Thompson, R. E. Huppler and R. E. Gilbert. The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. *J Pharmacol Exp Ther* **197:** 517-532, 1976.
5. Moroni, F., D. L. Cheney and E. Costa. Inhibition of acetylcholine turnover in rat hippocampus by intraseptal injection of β-endorphin and morphine. *Naunyn Schmiedebergs Arch Exp Pathol Pharmacol* **299:** 149-153, 1977.
6. Moroni, F., D. L. Cheney and E. Costa. The turnover rate of acetylcholine in brain nuclei of rats injected intraventricularly and intraseptally with alpha and beta endorphin. *Neuropharmacology* **17:** 191-196, 1978.
7. Paxinos, G. and C. Watson. *The Rat Brain in Stereotaxic Coordinates*. New York: Academic Press, 1982.
8. Robinson, S. E., D. L. Cheney and E. Costa. The regulation of hippocampus cholinergic neurons by adrenergic mechanisms. In: *Catecholamines: Basic and Clinical Frontiers*, edited by E. Usdin, I. J. Kopin and J. D. Barchas. Oxford: Pergamon Press, 1979, pp. 1077-1079.
9. Robinson, S. E., D. M. Sorenssen, P. L. Wood and J. W. Commission. Dopaminergic control of the septal-hippocampal cholinergic pathway. *J Pharmacol Exp Ther* **208:** 476-479, 1979.
10. Wood, P. L., D. L. Cheney and E. Costa. An investigation of whether septal gamma-aminobutyrate containing interneurons are involved in the reduction in turnover rate of acetylcholine elicited by substance P and β-endorphin in the hippocampus. *Neuroscience* **4:** 1479-1484, 1979.
11. Wood, P. L., L. M. Statland and A. Raekham. Opiate receptor regulation of acetylcholine metabolism: Role of mu, delta, kappa and sigma narcotic receptors. In: *Dynamics of Neurotransmitter Function*, edited by I. Hanin. New York: Raven Press, 1984, pp. 99-107.