PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 154, 540-542 (1977)

Emetic and Hyperthermic Effects of Centrally Injected Methionine-Enkephalin in Cats (39713)

WESLEY G. CLARK

Department of Pharmacology, Southwestern Medical School, The University of Texas Health Science Center at Dallas, 5323 Harry Hines Blvd., Dallas, Texas 75235

Methionine- and leucine-enkephalins are endogenous pentapeptides which have been isolated from both pig (1) and bovine (2) brain and which have been shown to cause opiate-like effects such as analgesia (3-6) and physical dependence (7) in rats and mice. Morphine and related agents cause both emesis (8, 9) and hyperthermia (10) when injected into the cerebral ventricles of cats. This study was performed to determine whether methionine-enkephalin can also cause these effects in the cat and, if so, to learn if naloxone is an effective antagonist.

Materials and methods. Nine adult cats were prepared with third cerebral ventricular cannulas (11) and retroperitoneal thermocouples (12). All glassware was rendered nonpyrogenic by sterilization in dry heat at over 200° for at least 2 hr. Commercial, nonpyrogenic, disposable syringes and needles were used for injections. Environmental temperature was maintained at 22 ± 1°. The initial injection each day was given at 10:00 AM (±5 min), and the average of temperature readings recorded automatically at 9:30, 9:45, and 10:00 AM was used as the baseline from which changes were measured. Naloxone hydrochloride (Endo Laboratories) and morphine sulfate (Mallinckrodt) were dissolved in commercial, nonpyrogenic 0.9% saline solution shortly before use. Doses of these drugs refer to their salts. Methionine-enkephalin (Calbiochem) was prepared similarly except that it was necessary to acidify the saline to pH 3-4 with hydrochloric acid to dissolve enough of the enkephalin for injections of 1 and 2 mg. Saline solutions of similar acidity have been shown not to appreciably alter body temperature within 4-5 hr after injection (13). All agents were injected in a volume of 50 μl. The cannulas were flushed with 100 μl of saline solution after recovery from any hyperthermic response or on the day following a drug injection. In crossover studies of the effect of the enkephalin or morphine in animals pretreated in random order with and without naloxone, saline or naloxone was given at 10:00 AM and the enkephalin or morphine was given 30 min later. Enkephalin and morphine injections were spaced at least 72 hr apart to avoid possible development of tolerance. This interval between injections of morphine is sufficient to avoid tolerance in the cat (10).

Results. Doses of methionine-enkephalin of 0.5 mg or less did not cause emesis (Table I). However, 1-2 mg evoked vomiting on at least one occasion in all cats tested. In only 2 of 19 tests was emesis not evoked. In crossover studies with 2 mg of enkephalin, vomiting did not occur if the cats were pretreated with naloxone (100-250 μg). These doses of naloxone alone did not cause emesis.

A dose of 1-2 mg of methionine-enkephalin was likewise required to evoke shivering and definite elevations of body temperature (Table II). Responses began within 10 min and lasted approximately 2 hr, with the maximum rise occurring 30-45 min after injection. In one crossover study with intervals of 5-6 days between tests, 100 μg of naloxone reduced the maximum increase of body temperature in three of five cats. In a similar study with an interval of 12 days between tests, 250 μg of naloxone reduced

TABLE I. EMETIC RESPONSES TO THIRD VENTRICULAR INJECTIONS OF METHIONINE-ENKEPHALIN.

Dose (mg)	Number of cats	Responses/ tests	Latency (min)	
			Mean	Range
0.5	3	0/3	–	–
1.0	7	8/9	4	1-6
2.0	6	9/10 (0/10)[a]	5	3-8

[a] Naloxone (100-250 μg) injected into the third ventricle 30 min before enkephalin.

ISSN 0037-9727

TABLE II. HYPERTHERMIC RESPONSES TO THIRD VENTRICULAR INJECTIONS OF METHIONINE-ENKEPHALIN AND MORPHINE SULFATE.

	Dose (mg)	Number of cats	Mean (range) maximum increase in body temperature (°)	
			Pretreatment	
			Saline	Naloxone
Methionine-enkephalin	0.5	3	0.4 (0.2–0.8)	–
	1.0	7	0.8 (0.3–1.1)	–
	2.0	5	1.2 (0.5–2.0)	0.8 (0.2–2.1)[a]
	2.0	5	1.2 (0.6–1.7)	0.7 (0.1–1.3)[b]
Morphine sulfate	10.0 μg	6	1.0 (0.3–2.0)	0.2 (0.0–0.9)[b]

[a] Naloxone (100 μg) 30 min before enkephalin.
[b] Naloxone (250 μg), $P < 0.05$ by paired t test.

the maximum response of all five cats. This latter dose of naloxone completely prevented the usual hyperthermic response to 10 μg of morphine sulfate in five of six cats (Table II). Neither naloxone alone nor the acidified saline vehicle appreciably altered body temperature. Other effects noted after enkephalin injections were mydriasis and vocalization.

Discussion. The above results demonstrate two central morphine-like actions of methionine-enkephalin in the cat. Inhibition of the responses by naloxone indicates that the enkephalin acted via opiate receptors. Naloxone has previously been reported to inhibit the emetic response to centrally administered morphine in the cat (9). The hyperthermic action of enkephalin seemed relatively more resistant to antagonism by naloxone than that of morphine, which was usually completely prevented by such pretreatment. Aside from emesis and hyperthermia, other effects elicited by morphine such as mydriasis and vocalization (14) were also seen after enkephalin administration. Based on the amount of agonist required to cause comparable increases in body temperature, 1–2 mg of methionine-enkephalin was about as potent as 10 μg of morphine sulfate. It has been estimated that at least 40 times as much enkephalin as morphine is required for comparable analgesic responses in the rat (6). The duration of hyperthermic action of the enkephalin was much shorter than that of 10 μg of morphine in the cat (10). The low potency and short duration of action of enkephalin was probably the result of rapid enzymatic degradation (6). It is unlikely that endogenous enkephalins function normally to control body temperature since pure opiate antagonists have been shown not to markedly alter body temperature in nondependent animals, even under conditions of thermal stress (15).

Summary. Third cerebral ventricular administration of 1–2 mg of methionine-enkephalin caused both emesis and hyperthermia in cats. The emetic response was prevented, and the hyperthermic response was reduced by 100–250 μg of naloxone given intraventricularly 30 min before enkephalin. These results provide further evidence that enkephalins can act on opiate receptors in the brain.

This investigation was supported by USPHS Research Grant NS 08618. I would like to thank Ms. Jean Ann Robins for her technical assistance.

1. Hughes, J., Smith, T. W., Kosterlitz, H. W., Fothergill, L. A., Morgan, B. A., and Morris, H. R., Nature (London) **258,** 577 (1975).
2. Simantov, R., and Snyder, S. H., Life Sci. **18,** 781 (1976).
3. Belluzzi, J. D., Grant, N., Garsky, V., Sarantakis, D., Wise, C. D., and Stein, L., Nature (London) **260,** 625 (1976).
4. Büscher, H. H., Hill, R. C., Römer, D., Cardinaux, F., Closse, A., Hauser, D., and Pless, J., Nature (London) **261,** 423 (1976).
5. Graf, L., Szekely, J. I., Ronai, A. Z., Dunai-Kovacs, Z., and Bajusz, S., Nature (London) **263,** 240 (1976).
6. Chang, J.-K., Fong, B. T. W., Pert, A., and Pert, C. B., Life Sci. **18,** 1473 (1976).
7. Wei, E., and Loh, H., Science **193,** 1262 (1976).
8. Borison, H. L., J. Physiol. **147,** 172 (1959).
9. Costello, D. J., and Borison, H. L., Fed. Proc. **35,** 309 (1976).
10. Clark, W. G., and Cumby, H. R., Neurosci.

Absts. **2,** 559 (1976).
11. McCarthy, L. E., and Borison, H. L., Anat. Rec. **155,** 305 (1966).
12. Clark, W. G., and Moyer, S. G., J. Pharmacol. Exp. Ther. **181,** 183 (1972).
13. Clark, W. G., and Cumby, H. R., Neuropharmacology **14,** 313 (1975).
14. Feldberg, W., and Shaligram, S. V., Brit. J. Pharmacol. **46,** 602 (1972).
15. Goldstein, A., and Lowery, P. J., Life Sci. **17,** 927 (1975).

Received December 28, 1976. P.S.E.B.M. 1977, Vol. 154.