Proc. Natl. Acad. Sci. USA
Vol. 74, No. 2, pp. 774–776, February 1977
Medical Sciences

β-Endorphin: Behavioral and analgesic activity in cats

(morphine/naloxone/enkephalin/pinch test/jaw-opening reflex)

MARIO MEGLIO*, YOSHIO HOSOBUCHI*, HORACE H. LOH†, JOHN E. ADAMS*, AND CHOH HAO LI‡

* Department of Neurosurgery, † Langley Porter Neuropsychiatric Institute and Department of Pharmacology, and, ‡ Hormone Research Laboratory, University of California, San Francisco, Calif. 94143

Contributed by Choh Hao Li, December 10, 1976

ABSTRACT **β-Endorphin has been shown to possess potent behavioral and antinociceptive activities when administered intraventricularly in cats. On a molar basis, β-endorphin is 72–96 times more potent than morphine and its actions are blocked by the specific opiate antagonist, naloxone.**

β-Endorphin, an untriakontapeptide isolated from camel pituitary glands (1), has been synthesized (2) and shown to possess opiate-like activity in receptor binding assays and in the guinea pig ileum bioassay (1–3). The analgesic activity of the synthetic peptide has been tested in rodents by intracerebral injection and found to be 18–33 times more potent than morphine, when compared on a molar basis, as shown by the tail-flick test, the hot plate test, and the acetic acid-induced writhing method in mice and the ice water-induced shake response in rats (4). In addition, β-endorphin has also been tested in mice via intravenous administration and found to be 3–4 times more potent than morphine, on a molar basis, in its analgesic effect (5). The present study was undertaken to determine the analgesic and behavioral effects of the peptide administered intraventricularly in the awake, unrestrained cat.

MATERIALS AND METHODS

Met-enkephalin was purchased from Bachem Laboratories (Marina del Reyes, Calif). β-Endorphin was synthesized as previously described (2). Naloxone hydrochloride was purchased from Endo Laboratories (Garden City, N.Y.). Morphine sulfate was purchased from Mallinckrodt Chemical Works (St. Louis, Mo.). Experiments were carried out on five cats chronically prepared with cannulas (0.6 mm in-shaft diameter) placed stereotactically in the third ventricle and fixed to the skull by means of acrylic resin. The extent of analgesia was determined by stimulating tooth pulp (6–10) and by measuring the jaw-opening reflex (JOR) (11). With the latter method, low threshold (2–7 V; 0.2 msec) and relatively constant reflex amplitudes were obtained as reported previously (12). All the surgical procedures were performed under sterile conditions with pentobarbital (30 mg/kg, intraperitoneal) anesthesia.

The analgesic effect of intraventricularly administered β-endorphin was also tested by pinches applied with a small hemostat or tooth forceps to the tail, the four limbs, and the ears. During the early stages of testing, pinch intensity was barely adequate to elicit an orienting reaction, vocalization, and withdrawal. Later, if β-endorphin appeared to block such responses, pinch intensity was progressively increased to a level which in the normal cat would elicit vigorous escape attempts, attack, and hissing. The pharmacological agents tested were considered to have an unequivocal analgesic effect only if the elevation in the threshold of the JOR was accompanied by alterations of behavioral signs after application of strong natural noxious stimuli (pinches) to the skin.

After recovering from the effects of surgery (at least 48 hr) the cats were placed in a cage to be tested. The analgesic properties of β-endorphin were tested in doses of 6.25, 12.5, 25, and 50 μg to determine its minimum effective dose (MED). When the MED was determined, naloxone (0.2 or 0.8 mg/kg) was given intraperitoneally to test its specific antagonistic action on opiate-like analgesia induced by β-endorphin. Morphine was given in doses of 25, 50, 100, 150, and 200 μg intraventricularly to determine its MED for analgesia. Met-enkephalin was given in a dose of 400 μg. All peptides tested, as well as morphine sulfate, were injected intraventricularly in 0.5 ml of isotonic saline per cat. After the completion of pharmacological testing, the cats were sacrificed and their brains were perfused with formaldehyde solution. Anatomical preparations were made to verify the location of the cannula in each animal.

RESULTS

Intraventricular injection of 6.25 μg of β-endorphin caused no behavioral changes or evidence of analgesia with the JOR or pinch test during 3 hr of observations (Table 1). However, 15–20 min after administration of 12.5 μg of β-endorphin, all cats showed an alteration of their behavior as follows: (*i*) fine tremors of the head accompanied by sudden quick head movements; (*ii*) mild excitation; (*iii*) fixation of eyes into space or on an object (cats barely responded to external noises—shaking of the cage, tapping of the floor, calling, etc.—and were hardly distracted from their apparent fixation on phantom objects; they changed their direction of gaze from time to time, but they always appeared to have "visual hallucinations"); and (*iv*) pupillary dilatation varied in intensity from one cat to the other, although fixation of eyes into space was prominent in all cats tested. The altered behavior was evident for about 1 hr, after which the animals appeared to return to their normal behavioral state. In none of the animals was intraventricular injection of 12.5 μg of β-endorphin followed by a significant alteration in JOR or response to pinch test.

Intraventricular injection of 25 μg of β-endorphin induced, within 15–20 min, alteration of behavior in all cats as described above. In general, the alterations were somewhat more pronounced and longer lasting than those seen after administration of 12.5 μg of the peptide. In all animals the intraventricular administration of β-endorphin was followed by a significant rise of the threshold for the JOR. Such an increase in threshold was always concomitant with or came after the first signs of behavioral change. The maximal increase of the JOR threshold was reached about 60 min after peptide administration (Fig. 1). The threshold at this point was 3 or 4 times greater than the control value. At the same time, the cats did not show any sign of discomfort or pain in response to forcible skin pinch by tooth

Abbreviations: JOR, jaw-opening reflex; MED, minimum effective dose.

Table 1. Summary of MED for analgesia determinations with intraventricularly administered β-endorphin

Cat	β-Endorphin dose*			
	6.25 μg	12.5 μg	25.0 μg	50.0 μg
1	—	– (B)	+ (B)	+ (B)
2	—	– (B)	+ (B)	+ (B)
3	—	– (B)	+ (B)	
4	—	– (B)	+ (B)	
5	—	– (B)	+ (B)	

* (B) indicates the presence of behavioral alteration.

forceps. The animals were freely mobile in the cage and did not show any evidence of paralysis or catatonia. The threshold of the JOR returned to the control value in about 2 hr after the peak time, or approximately 3 hr after the time of administration of β-endorphin. The behavioral alterations, however, disappeared somewhat earlier.

Only two cats were tested with 50 μg of β-endorphin, because the above experiments clearly indicated the MED of the peptide to be 25 μg. At this high dose (50 μg), more pronounced behavioral changes and a greater increase in JOR threshold were observed (Fig. 2). The time course of the disappearance of the behavioral changes and return of the control JOR threshold were also prolonged.

In all five cats, the MED of β-endorphin (25 μg) was administered intraventricularly; after the peak analgesic effect of the peptide was reached as determined by JOR, naloxone was given intraperitoneally, 0.2 mg/kg in three cats and 0.8 mg/kg in two. One of the cats that received naloxone at 0.2 mg/kg did not clearly show alteration or reduction in the elevated JOR threshold or behavior changes induced by β-endorphin. In the four other cats, a significant decrease in JOR threshold was observed immediately after the administration of naloxone and lasted about 20 min, after which the animal's behavior returned to normal. As the JOR threshold began to rise again, a clear behavioral change reappeared. JOR threshold and behavior returned to the control state in about 1 hr. In two cats, which received naloxone at 0.8 mg/kg, normalization of behavior and decrease in elevated JOR threshold were observed within a few minutes (Fig. 3). The effect of the naloxone was again observed for 20–30 min, and again JOR threshold elevation and behavioral changes reappeared, although not to as great an extent nor for as long as with naloxone at 0.2 mg/kg.

When morphine sulfate was tested, at the lower doses (25, 50, and 100 μg) only behavioral alterations resembling those seen after β-endorphin administration occurred. With morphine sulfate, however, the cats were more excited and agitated. They vocalized frequently, making JOR determination rather difficult. Only at morphine doses of 150 and 200 μg were we able to see a significant increase of JOR as well as profound behavioral alterations.

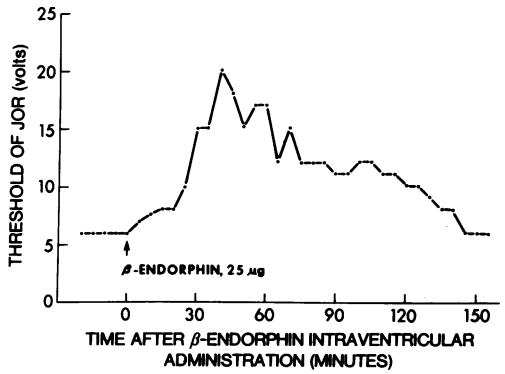

FIG. 1. JOR threshold changes after intraventricular injection of 25 μg of β-endorphin.

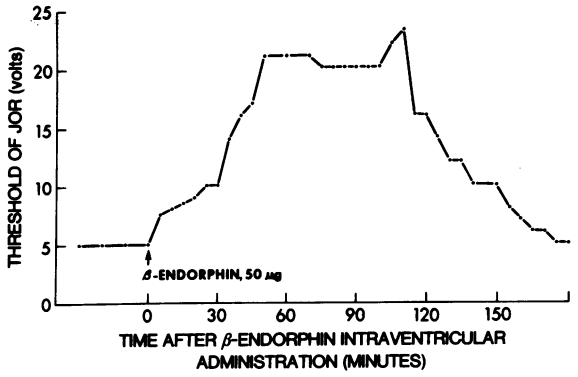

FIG. 2. JOR threshold changes after intraventricular injection of 50 μg of β-endorphin.

Intraventricular administrations of 100, 200, and 400 μg of Met-enkephalin failed to produce an analgesic effect. There was no alteration in animal behavior, JOR, or response to pinch test during a 3-hr observation period.

DISCUSSION

β-Endorphin administered intraventricularly at doses of 25 and 50 μg in the cat produced a dose-related inhibition of JOR and of response to nociceptive stimuli applied to the skin. This antinociceptive action of β-endorphin was accompanied by significant alterations in the animal's behavior. In fact, the behavioral alteration was observed at a dose level of 12.5 μg without significant change in JOR or response to nociceptive stimuli. The antinociceptive effect and behavioral changes induced by β-endorphin lasted about 2 hr and resembled those induced by morphine sulfate except that with morphine the cats were more excitable. These effects induced by 25-μg doses of β-endorphin were promptly partially reversed by intraperitoneally administered naloxone at 0.8 mg/kg.

It is interesting to note that during this experiment a recovery period of at least 72 hr was allowed for each animal between drug administration and pharmacological testing. This recovery period was essential because, if β-endorphin was administered within less than 24 hr, there were observable alterations in the animal's behavior but no antinociceptive effect of the peptide.

Met-enkephalin, reported by others to have some analgesic activity of short duration in rodents (4, 13), did not produce any

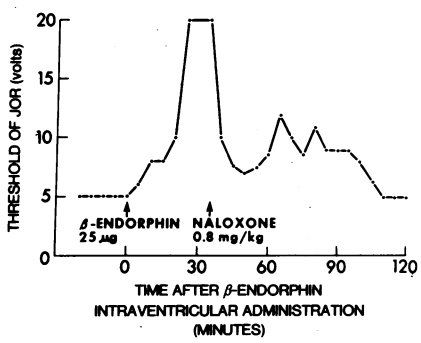

FIG. 3. JOR threshold changes after intraventricular injection of 25 μg of β-endorphin followed by intraperitoneal injection of naloxone, 0.8 mg/kg.

alteration in behavior, JOR, or response to nociceptive cutaneous stimuli at relatively high doses in the cat[§]. These data support the contention that *in vitro* assays, although highly accurate in predicting the analgesic potentials of opiate alkaloids, do not appear to be quantitative estimators for the *in vivo* activities of opiate-like peptides.

This investigation was supported in part by U.S. Public Health Service Training Grant NS-05593 from the National Institute of Neurological Diseases and Stroke, the Lorenzo Lo Memorial Pain Research Fund, and the Guggenheim Memorial Fund as well as NIDA Grants DA-01314 and DA-00564 and NIH Grant GM-2907.

1. Li, C. H. & Chung, D. (1976) *Proc. Natl. Acad. Sci. USA* **72**, 1145–1148.
2. Li, C. H., Lemaire, S., Yamashiro, D. & Doneen, B. A. (1976) *Biochem. Biophys. Res. Commun.* **71**, 19–25.

[§] In this connection, it may be noted that, as quoted by Bradbury *et al.* (14) W. F. Feldberg observed the analgesic activity of β-endorphin when injected directly into the third ventricle of the cat.

3. Cox, B. M., Goldstein, A. & Li, C. H. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1821–1823.
4. Loh, H. H., Tseng, L. F., Wei, E. & Li, C. H. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2895–2898.
5. Tseng, L. F., Loh, H. H. & Li, C. H. (1976) *Nature* **263**, 239–240.
6. Bessou, P., Gauthier, J. & Pages, B. (1970) *C. R. Seances Soc. Biol. Ses. Fil.* **164**, 1845–1850.
7. Brookhart, J. M., Livingston, W. R. & Haugen, F. P. (1953) *J. Neurophysiol.* **16**, 634–642.
8. Pfaffman, C. (1939) *J. Physiol. (London)* **97**, 207–219.
9. Windle, W. F. (1927) *J. Comp. Neurol.* **43**, 347–356.
10. Anderson, D. J., Hannam, A. G. & Mathews, B. (1970) *Physiol. Rev.* **50**, 171–185.
11. Mitchell, C. L. (1964) *J. Pharmacol. Exp. Ther.* **146**, 1–6.
12. Oliveras, J. L., Woda, A., Guilbaud, G. & Besson, J. M. (1974) *Brain Res.* **72**, 328–331.
13. Bellinzi, J. D., Grant, N., Garsky, V., Sarantakis, D., Wise, C. D. & Stein, L. (1976) *Nature* **260**, 626–627.
14. Bradbury, A. F., Smyth, D. G., Snell, C. R., Birdsall, N. J. M. & Hulme, E. C. (1976) *Nature* **260**, 793–795.