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β-Endorphin is a potent analgesic agent

(tail-flick, hot-plate, writhing, and wet shake tests/morphine/enkephalin/lipotropin)

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ABSTRACT **β-Endorphin, an opiate-like peptide, has potent antinociceptive properties when it is administered directly into the brain and assayed in the tail-flick, hot-plate, and writhing tests in mice and in the wet shake test in rats. On a molar basis, β-endorphin is 18 to 33 times more potent than morphine and its actions are blocked by the specific opiate antagonist, naloxone hydrochloride. The activity of β-endorphin *in vivo* is also compared to other peptides that show opiate-like activity in assays *in vitro*.**

The existence of endogenous ligands for the opiate receptor in the brain has been suggested by Collier (1) and Goldstein (2). In searching for such ligands, Terenius and Wahlstrom (3) and Hughes (4), using the receptor binding assay and bioassays with mouse vas deferens and guinea pig ileum, independently found opiate-like substances in the brain. Subsequently, Hughes *et al.* (5) purified and characterized two opiate-like peptides, termed enkephalins, which have the following amino acid sequences: H-Tyr-Gly-Gly-Phe-Met-OH and H-Tyr-Gly-Gly-Phe-Leu-OH. Other workers (6, 7) have confirmed the results of Hughes *et al.* Concurrently, Goldstein and his associates (8, 9) found opiate-like materials in a crude preparation of corticotropin (ACTH). Synthetic corticotropin and α-melanotropin lacked similar activity. Upon purification and characterization, the corticotropin contaminant was shown to be a peptide with a molecular weight of approximately 1750. Recently, Li and Chung (10) isolated and determined the sequence of an untriakontapeptide from camel pituitary gland. This peptide, named β-endorphin, has been synthesized (11) and shown to possess opiate-like activity in receptor binding assays and in the guinea pig ileum bioassay (10–12).

In the isolation and identification of opiate-like peptides from brain and pituitary tissue, the criteria for specific activity has been based on bioassays *in vitro*. Information on the pharmacological properties of these peptides *in vivo* is lacking, partly due to the scarcity of purified or synthetic materials. In this article, we report on the relative analgesic properties of β-endorphin and related peptides when assayed in the mouse and rat *in vivo*.

MATERIALS AND METHODS

Male ICR mice weighing 25–30 g (Simonsen Laboratories, Gilroy, Calif.) and male Sprague-Dawley rats weighing 250–350 g were used in all the experiments.

Methionine-enkephalin was purchased from Bachem Laboratories (Marina del Reyes, Calif). β-Endorphin was synthesized as previously described (11). β-Lipotropin (LPH) was isolated from sheep pituitary glands by the procedure described by Li *et al.* (13). Naloxone·HCl was a gift from Endo Laboratories (Garden City, N.Y.). Morphine sulfate was purchased from Mallinckrodt Chemical Works (St. Louis, Mo.). Sodium pentobarbital (Diabutal) was prepared for injection by diluting with distilled water to 25 mg/ml. Morphine pellets containing 75 mg of morphine base were formulated according to Gibson and Tingstad (14).

Assay for antinociceptive activity

The antinociceptive or analgesic properties of β-endorphin and morphine were assessed by the tail-flick method (15), the hot-plate method (16), and the acetic acid-induced writhing method (17) in mice and by the ice water-induced wet shake response in rats (18). All peptides tested and morphine sulfate were injected intracerebrally (i.c.) in a volume of 5 μl per mouse (19) or 1 μl per rat (18).

Hot-Plate and Tail-Flick Response in Mice. To evaluate these responses, a control latency (T_0) was obtained from the mean of three latencies determined prior to drug injection, and the test latencies (T_1) were determined at various times after injection for each animal. "Percent analgesia" was calculated as $(T_1 - T_0)/(T_2 - T_0) \times 100$, where the cut-off time (T_2) for the hot-plate and tail-flick test were 60 and 15 sec, respectively.

With 2-fold increase in latency of reaction time as a quantal index of inhibition, the median antinociceptive dose (AD_{50}) and 95% confidence limits were calculated according to the method of Litchfield and Wilcoxon (20). At least eight animals were tested at each dose with three to five dose levels used for determining the AD_{50}.

Writhing Test in Mice. When "analgesia" was measured by the writhing method (17), acetic acid (0.1 ml/10 g of body weight of 0.6%) was administered intraperitoneally (i.p.) 5 min after i.c. injection of β-endorphin. The number of writhes was counted for 15 min. To determine the AD_{50} for writhing, a quantal index of inhibited response was defined as any mouse that writhed less than twice after drug administration.

Wet Shake Response in Rats. The inhibitory effects of β-endorphin on repetitive shaking movements, induced by immersion of anesthetized rats in ice water, were also studied. This experimental model, which has been described in detail (18), is a sensitive bioassay for detecting central opiate activity *in vivo*. Animals were anesthetized with sodium pentobarbital, 50 mg/kg i.p. Thirty minutes later, β-endorphin, dissolved in distilled water, was injected bilaterally, at a volume of 0.5 μl per hemisphere, into the periaqueductal gray region of the rat brain. Five minutes after injection, the animal was immersed in ice water and the number of shaking movements was counted for 5 min. The periaqueductal gray was chosen for study because recent microinjection experiments show that this brain site is highly sensitive to the pharmacological actions of morphine (21–23).

Assessment of physical dependence

To study the ability of β-endorphin to induce physical dependence, a device known as an osmotic minipump (Alzet™

Abbreviations: i.p., intraperitoneally; i.c., intracerebrally; LPH, β-lipotropin; AD_{50}, median antinociceptive dose.

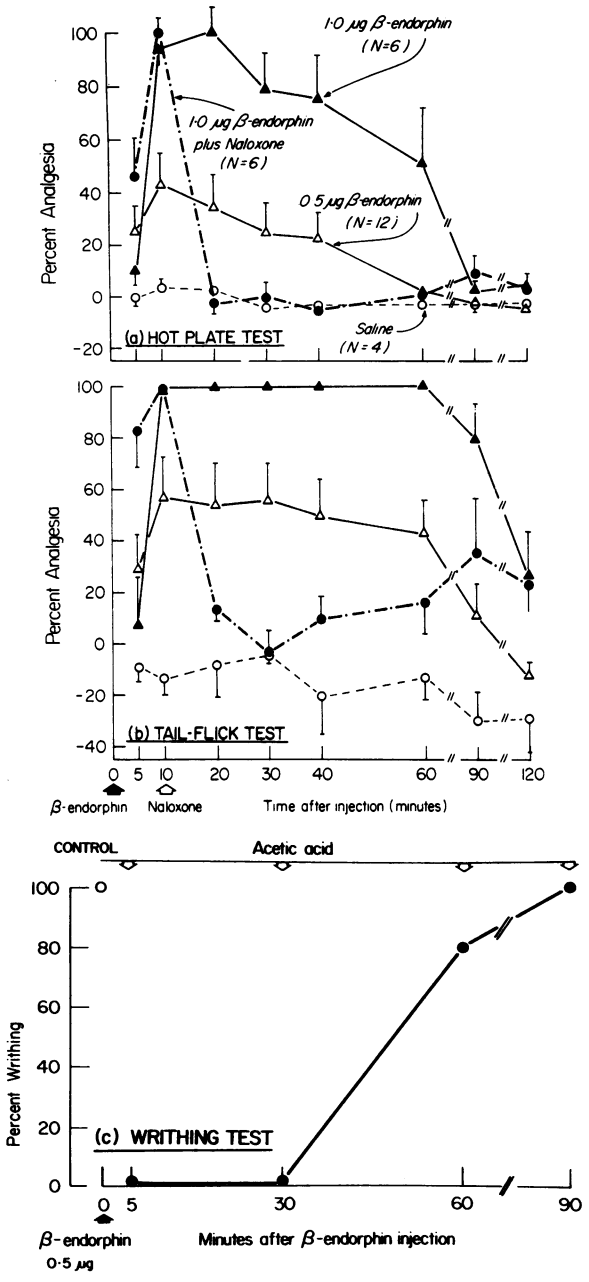

FIG. 1. Antinociceptive effects of β-endorphin in the (a) hot-plate test, (b) tail-flick test, and (c) writhing test and its reversal by naloxone. In (a) and (b) β-endorphin was injected i.c. at 0 time (↑). Naloxone (1 mg/kg subcutaneously) was injected at 10 min after β-endorphin. N = number of mice studied. In control mice, 5 μl of saline was injected intracerebrally. The vertical bars indicate the SEM. In (c) four groups of mice, N = 5–8 mice per group, were injected with β-endorphin 0.5 μg i.c. After challenge with acetic acid (0.6%, 0.1 ml/10 g of body weight, injected i.p.), the writhing responses were counted for 10 min. A quantal index of inhibition was defined as less than two writhing movements.

generic delivery system, Alza Corp., Palo Alto, Calif.) was used. The minipump, which is a system for infusing small volumes at constant rates (1.40 ± 0.4 μl/hr for approximately 5 days), was used to deliver drugs directly into the brain.

Animals were anesthetized with sodium pentobarbital, 50 mg/kg i.p., and L-shaped stainless steel cannulas, made from 21-gauge disposable needles and filed to a predetermined length, were implanted into the frontal cortex or periaqueductal gray. The implanted cannula, filled previously with distilled water, was secured to the skull with dental cement. The minipump, filled with the desired drug solution, was inserted subcutaneously between the scapulae in the anesthetized animal, and a 21-gauge stainless steel tube protruding from the minipump was then coupled to the brain cannula with plastic tubing. To avoid dislodgement of the pump by the animal, the scalp wound was closed with sutures so that the entire infusion unit was enclosed under the skin.

After 70 hr of drug infusion into the brain, animals were weighed, placed in 1-gallon glass jars, and after a 10- to 15-min adjustment period, challenged with the specific opiate antagonist, naloxone hydrochloride, 10 mg/kg i.p. The resultant withdrawal behavior was then observed under standardized procedures, as described previously (24). Leaping attempts to escape from the glass jar, "wet dog shakes," and teeth chattering are examples of distinctive abstinence behavior that occur in the dependent animal after the administration of opiate antagonists (24). If a rat made two or more escape attempts from the jar, or had three or more "wet dog shakes," or made grinding noises with its teeth within 15 min after injection of naloxone, it was considered to have undergone precipitated withdrawal and was classified as manifesting the particular withdrawal sign (24, 25).

RESULTS AND DISCUSSION

Pharmacological properties of β-endorphin

β-Endorphin, at doses of 0.5 μg and 1.0 μg administered centrally per mouse, produced a dose-related inhibition of the tail-flick and hot-plate responses of mice to nociceptive stimuli (Fig. 1a and b). This effect lasted 60–90 min, depending on the doses used, and closely resembles the duration of morphine action in the same tests. The antinociceptive action of β-endorphin in these two tests *in vivo* is fully reversed by naloxone, a pure narcotic antagonist (Fig. 1). In the writhing assay, β-endorphin produced a dose-related inhibition of the writhing responses of mice to acetic acid injections, an effect which also lasted for 60–90 min (Fig. 1c). Pretreatment of mice with naloxone·HCl (1 mg/kg subcutaneously) 5 min before the injection of β-endorphin completely abolished the inhibitory effect of β-endorphin on writhing. Finally, in the wet shake test in rats, β-endorphin again produced a dose-related inhibition of the shaking response to ice water, and this inhibitory effect was readily antagonized by naloxone administered, 5 mg/kg i.p., 15 min before β-endorphin. Thus, in four tests for central antinociceptive activity, β-endorphin acts like an opiate agonist *in vivo*. Furthermore, these agonist actions of β-endorphin were reversed by naloxone (Fig. 2).

In order to determine the addictive liability of this new "peptide analgesic," the osmotic minipump, as described above, was used to deliver β-endorphin directly into the periaqueductal gray fourth ventricular spaces of rat brain at a rate of 1.4 μl/hr. A solution of β-endorphin (0.67 μg/μl) was infused for 70 hr. When challenged with naloxone, almost all the animals manifested a typical morphine-like withdrawal syndrome.

Relative potency of β-endorphin to related peptides and morphine

A quantitative comparison of analgesic potency between β-endorphin and morphine in the three mouse bioassays is shown in Table 1. When potency is compared on a molar basis, β-endorphin was approximately 18–33 times more active than morphine sulfate.

In order to gain additional insight into the relationship be-

Table 1. AD_{50}* of β-endorphin and morphine in mice

Tests	β-Endorphin	Morphine sulfate	Potency ratio
Writhing	0.92 (0.48–1.56)	17.92 (10.76–27.52)	19.5
Hot-plate	2.32 (1.4–3.96)	40.68 (32.28–51-44)	17.5
Tail-flick	1.52 (0.88–2.64)	50.24 (43.04–58.60)	33.0

The molecular weights of 3438 and 334 were used for β-endorphin and morphine sulfate, respectively.
* AD_{50} in nmol/kg i.c. (95% confidence limits).

tween peptide structure and analgesic activity, peptides related to β-endorphin were tested in mice. β-Lipotropin, a 91 amino acid peptide (13, 26) containing β-endorphin within its structure [β-LPH(61–91)], was at least 80 times less active than β-endorphin. The nonapeptide, β-LPH(61–69), at doses up to 200 nmol/kg did not show any significant antinociceptive properties in these tests. Methionine-enkephalin, a pentapeptide (5) containing the amino acid sequence 61–65 of β-LPH, produced a weak analgetic effect of short duration (less than 5–10 min) in all three tests when 50 μg (3.3 μmol/kg i.c.) was given to each mouse. Higher doses did not prolong the analgesic activity of methionine-enkephalin. These results with enkephalin are similar to the recent observations of Belluzzi *et al.* (27) of enkephalin activity in rats. Tryptic digestion of β-endorphin destroyed most of its analgesic potency, showing that the intact molecule was necessary for full activity.

The pharmacological potencies of β-endorphin and related peptides *in vivo* are of interest when compared to the relative activities of these peptides in bioassays *in vitro* such as stereospecific binding and guinea pig ileum. In opiate receptor binding assays, Cox *et al.* (12) showed that β-endorphin was two to three times more potent and enkephalin 1/5 as potent as normorphine. In the guinea pig ileum preparation, these authors (12) found that β-endorphin and enkephalin were approximately equipotent, but both were less active than normorphine. To account for the discrepancy in activity in the two bioassays

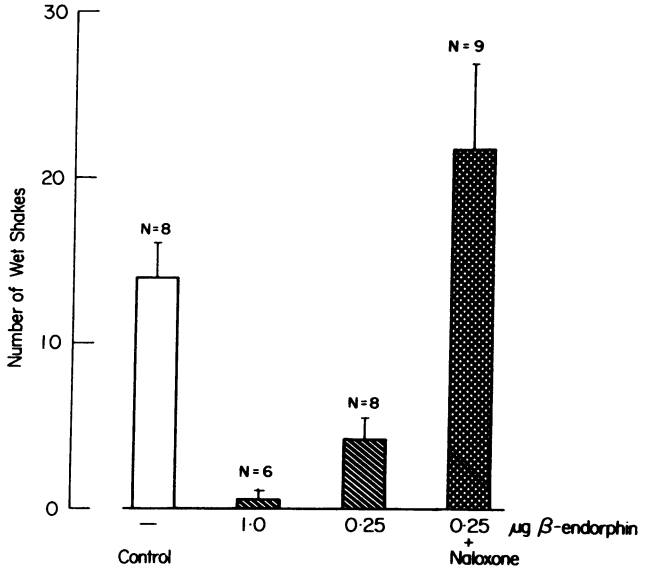

FIG. 2. Inhibitory effect of β-endorphin on wet shake behavior and its reversal by naloxone. Rats were anesthetized with sodium pentobarbital 30 min before the bilateral injection of β-endorphin into the periaqueductal gray in a volume of 0.5 μl per hemisphere. Five minutes after injection, rats were immersed in ice water and the number of wet shakes was counted for 5 min. Naloxone, 5 mg/kg subcutaneously, was injected 15 min before β-endorphin. Controls received saline injections.

in vitro, Cox *et al.* (12) suggested that permeability barriers to peptides may be present in the ileum preparation. In this study, the activity of β-endorphin *in vivo* was 18 to 33 times greater than morphine sulfate, whereas enkephalin was relatively inactive in the same test systems. It is very unlikely that the difference in central activity can be explained in terms of access of the peptides to the analgesic receptor or in terms of the rates of degradation of these peptides. Thus, the assays *in vitro*, although highly accurate in predicting the analgesic potencies of opiate alkaloids, do not appear to be quantitative estimators for the activities of opiate-like peptides *in vivo*.

In summary, we have shown that β-endorphin is a potent analgetic, being 18 to 33 times a more potent than morphine on a molar basis. Furthermore, we found that chronic, localized infusion of β-endorphin into the rat brain can induce morphine-like physical dependence. The present results raise an important question as to the biological role of β-lipotropin. The possibility that it serves as a prohormone to potent agonist (β-endorphin) as well as antagonist (β-melanotropin, Arg-enkephalin, etc.) peptides cannot be ignored (28–30).

Note Added in Proof. It has been reported that β-lipotropin(61–76) was active in the ileum assays *in vitro* [Guillemin, R., Ling, N. & Burgus, R. (1976) *Séances Acad. Sci. Ser. D.* **282**, 783–785]. In our experiments, 20–40 μg of β-lipotropin(61–76) was not active in all assay procedures *in vivo*, whereas 0.2 μg of β-endorphin elicited an analgesic effect.

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1. Collier, H. O. J. (1973) *Proc. 5th Int. Congr. Pharmacol., San Francisco, 1972*, ed. Cochin, J. (Karger, Basel), Vol. 1, pp. 65–76.
2. Goldstein, A. (1974) "Proc. Intl. Symp. Alcohol and Drug Res.," in *Biological and Behavioral Approaches to Drug Dependence*, eds. Cappel, H. & LeBlanc, A. E. (Addiction Research Foundation, Toronto, Canada), pp. 217–241.
3. Terenius, L. & Wahlstrom, A. (1974) *Acta Pharmacol. Toxicol. Suppl. 1*, **35**, 55.
4. Hughes, J. (1975) *Brain Res.* **88**, 295–308.
5. Hughes, J., Smith, T. W., Kosterlitz, H. W., Fothergill, L. A., Morgan, B. A. & Morris, H. R. (1975) *Nature* **258**, 577–579.
6. Pasternak, G. W., Goodman, R. & Snyder, S. H. (1975) *Life Sci.* **16**, 1765–1769.
7. Simantov, R. & Snyder, S. H. (1976) *Proc. Natl. Acad. Sci. USA*, **73**, 2515–2519.
8. Teschemacher, H., Opheim, K. E., Cox, B. M. & Goldstein, A. (1975) *Life Sci.* **16**, 1771–1776.
9. Cox, B. M., Opheim, K. E., Teschemacher, H. & Goldstein, A. (1975) *Life Sci.* **16**, 1777–1782.
10. Li, C. H. & Chung, D. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1145–1148.
11. Li, C. H., Lemaire, S., Yamashiro, D. & Doneen, B. A. (1976) *Biochem. Biophys. Res. Commun.*, in press.
12. Cox, B. M., Goldstein, A. & Li, C. H. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1821–1823.
13. Li. C. H., Barnafi, L., Chrétien, M. & Chung, D. (1966) *Excerpta*

Med. Int. Congr. Ser. **112,** 349–364.
14. Gibson, R. D. & Tingstad, J. E. (1970) *J. Pharm. Sci.* **59,** 426–427.
15. D'Amour, F. E. & Smith, D. L. (1941) *J. Pharmacol. Exp. Ther.* **72,** 74–79.
16. Antier, S. (1974) *Eur. J. Pharmacol.* **27,** 1–4.
17. Koster, R., Anderson, M. & DeBeer, E. J. (1959) *Fed. Proc.* **18,** 412.
18. Wei, E., Sigel, S. & Way, E. (1975) *J. Pharmacol. Exp. Ther.* **193,** 56–63.
19. Haley, T. J. & McCormick, W. G. (1957) *Br. J. Pharmacol.* **12,** 12–15.
20. Litchfield, J. T. & Wilcoxon, F. (1949) *J. Pharmacol. Exp. Ther.* **96,** 99–113.
21. Sharpe, L. G., Garnett, J. E. & Cicero, T. J. (1974) *J. Behav. Biol.* **11,** 303–313.
22. Jacquet, Y. & Lajtha, A. (1974) *Science* **185,** 1055–1057.
23. Herz, A., Albus, K., Metys, J., Shubert, P. & Teschemacher, H. T. (1970) *Neuropharmacology* **9,** 539–551.
24. Wei, E., Loh, H. H. & Way, E. L. (1973) *J. Pharmacol. Exp. Ther.* **184,** 398–403.
25. Wei, E., Loh, H. H. & Way, E. L. (1973) *J. Pharmacol. Exp. Ther.* **185,** 108–115.
26. Graf, L. & Li, C. H. (1973) *Biochem. Biophys. Res. Commun.* **30,** 1190–1206.
27. Belluzzi, J. D., Grant, N., Garsky, V., arantakis, D, Wise, C. D. & Stein, L. (1976) *Nature* **260,** 626–627.
28. Krivoy, W. A., Kroeger, D., Newman, T. & Zimmermann, E. (1974) *Eur. J. Pharmacol.* **27,** 339–345.
29. Zimmermann, E. & Krivoy, W. A. (1973) *Prog. Brain Res.* **39,** 383–394.
30. Ungar, G. & Ungar, A. L. (1976) *Fed. Proc.* **35,** 572.