Life Sciences Vol. 21, pp. 321-328, 1977 Pergamon Press
Printed in the U.S.A.

COMPARISON OF THE BEHAVIORAL EFFECTS OF β-ENDORPHIN AND ENKEPHALIN ANALOGS

E. T. Wei*, L. F. Tseng +, H. H. Loh+ and Choh Hao Li ++

*School of Public Health, University of California, Berkeley, CA 94720
+ LPNI & Dept. of Pharmacology, University of California, San Francisco, CA 94143
++ Hormone Research Laboratory, University of California, San Francisco, CA 94143

(Received in final form June 23, 1977)

Summary

The behavioral effects of β-endorphin, enkephalin analogs, morphine and etorphine were briefly compared. In the tail-flick test in mice and in the wet shake test in rats, β-endorphin and D-Ala^2-D-Leu^5-enkephalin had equal antinociceptive activity; D-Ala^2-Met-enkephalinamide and D-Leu^5-enkephalin were less active. The order of activity of the enkephalin analogs and opiate alkaloids for stimulating locomotor activity in mice paralleled their analgesic activities; β-endorphin, however, had only minimal stimulatory actions. Morphine sulfate, 50 μg injected into the periaqueductal gray, produced hyperactivity but this effect was not observed with etorphine or opioid peptides. By contrast, "wet dog" shakes was observed with the opioid peptides but not with either opiate alkaloid. These heterogenous behavioral responses, which were all antagonized by naloxone, indicate that multiple types of receptors mediate the effects of opiates in the central nervous system.

Fragments of β-lipotropin (LPH) and leucine-enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH) act as opiate agonists in in vitro assays (1-4). In vivo, large doses of methionine-enkephalin (LPH 61-65, H-Tyr-Gly-Gly-Phe-Met-OH) and leucine-enkephalin are required to produce analgesia of short duration (5,6). By contrast, β-endorphin (LPH 61-91) has potent analgesic and cataleptic activities in experimental animals (7-12). Rapid degradation of enkephalins by brain enzymes may in part account for its transient effects (13). Enkephalin analogs have been synthesized which resist degradation and some of these analogs, e.g. D-Ala^2-Met-enkephalinamide, have activity similar to that of β-endorphin (13,14). In this investigation, the behavioral effects of β-endorphin and enkephalin analogs are compared to

---

Supported in part by grants DA-00091, DA-01314, DA-00564 and GM-2907. H. H. Loh is a recipient of a NIMH Career Development Award, K2-DA-70554. This is Publication No. 7718 from the Department of Pharmacology, University of California, San Francisco.

determine if there are significant quantitative and qualitative differences between the short-chain and long-chain opiate peptides.

Methods

Male ICR mice weighing 25-30 g and male Sprague-Dawley rats weighing 180-350 g were used in all experiments. The antinociceptive properties of morphine sulfate and opiate peptides were assayed using the tail-flick responses of mice to radiant heat and the shaking responses of pentobarbital-anesthetized rats to ice water. These methods were described in previous publication (7,15). All drugs tested were dissolved in sterile saline and injected: (1) in mice, intracerebrally in a volume of 5 µl per mouse, (2) in the anesthetized rat, a single mid-saggital injection of 1 µl per rat, except for D-Leu5 -enkephalin which, because of its limited solubility, was injected in a volume of 2 µl, (3) in unanesthetized rats previously cannulated with a 21-gauge steel tube, the volume was 5 µl. Injections in rats were made into the periaqueductal gray-fourth ventricular spaces (PAG4), a brain area known to be selectively sensitive to morphine (16). The median antinociceptive dose (AD50) and 95% confidence limits were calculated according to the method of Litchfield and Wilcoxon (17). For studies of naloxone antagonism of opiate effects, naloxone HCl, 5 mg/kg i.p., was injected in rats 15-20 minutes after central administration of morphine, etorphine, β-endorphin or D-Ala^2-D-Leu^5-enkephalin. In mice, naloxone HCl was injected 1 mg/kg s.c., 5 minutes before intracerebral injections of D-Ala^2-D-Leu^5-enkephalin or D-Ala^2-Met-enekphalinamide.

Locomotor activity of mice was determined using an Electronic Motility Meter (Model Fc40, Motron Produktor, Stockholm, Sweden). Three mice were placed in a plastic cage (15 cm x 26 cm) and allowed to acclimatize to environmental conditions for 1 hour. Locomotor activity, estimated by frequency of crossing a light beam, was then recorded until values returned to pre-drug levels. The presence of catalepsy was measured by placing a rat with its forelegs on a bar 10 cm high. If the position was maintained for at least 20 seconds, the animal was considered cataleptic.

Drugs used were: morphine sulfate (Mallinckrodt Chemical Works, St. Louis, MO.), etorphine hydrochloride (gift from Reckitt and Colman, Hull, England), synthetic camel β-endorphin, also known as β_C-endorphin (18), enkephalin analogs (gift of Drs. R.J. Miller and S. Wilkinson, Burroughs Wellcome Co., Research Triangle Park, N.C.) and naloxone hydrochloride (gift of Endo Laboratories, Garden City, New York).

Results and Discussion

D-Ala^2-D-Leu^5-enkephalin, at doses of 11 ng to 43 ng caused a dose-related inhibition of the tail-flick response to heat stimuli. The antinociceptive response lasted 20 to 60 minutes depending on the dose used. The median antinociceptive doses of opiate peptides and morphine sulfate for the tail-flick response in mice are shown in Table 1. On a molar basis, D-Ala^2-D-Leu^5-enkephalin was as active as β-endorphin and was about 32 to 35 times more active than morphine; the other peptides were less potent. A similar result for the peptides was obtained in the wet shake test in rats (Fig. 1) where, in fact, the same line fits the dose-effect relationship for β-endorphin and D-Ala^2-D-Leu^5-enkephalin. In this test, however, morphine had approximately the same activity as β-endorphin and D-Ala^2-D-Leu^5-enkephalin. Thus, substitution of two D-amino acids on enkephalin produces an analog having the same antinociceptive activity as the untriakontapeptide, but the relative activity of these peptides to morphine depended on

TABLE I

AD_{50} of Morphine Sulfate and Opioid Peptides in Mice

	AD50* nmole per mouse	Potency Ratio Morphine Sulfate = 1
Morphine Sulfate	1.11(0.95-1.29)	1
D-Ala ^{2}-D-Leu ^{5}-Enkephalin	0.035(0.023-0.054)	31.7
D-Ala ^{2}-Met-Enkephalinamide	2.14(1.53-3.01)	0.5
D-Leu ^{5} -Enkephalin	179	0.006
β_c -Endorphin	0.032(0.020-0.049)	34.7

*AD_{50} and 95% Confidence Limits.

Inhibitory Effects of Enkephalin Analogs, β-Endorphin, Morphine and Etorphine on Wet Shake Behavior

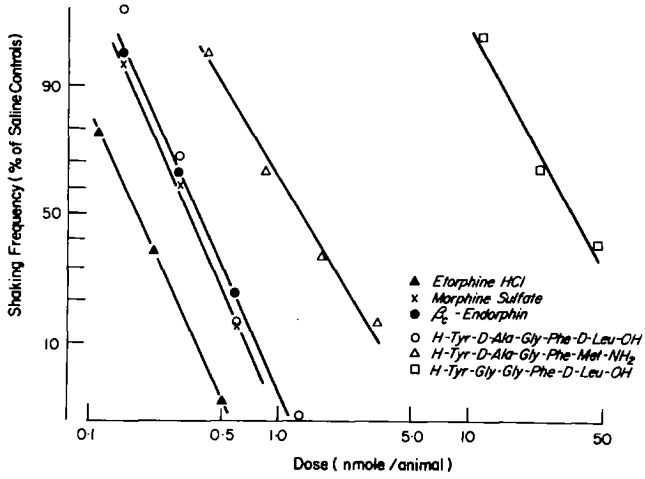

FIG. 1

Rats were anesthetized with sodium pentobarbital 30 minutes before a single mid-saggital injection of the chemical into the periaqueductal gray-fourth ventribular spaces. Ten minutes after injection, the animal was immersed in ice water and the number of shakes counted for 5 minutes. Animals receiving only saline injections shook 17.6± 3.0 times (N=16). Each point represents the mean value of at least 6 animals.

the bioassay.

The order of activity for these drugs in stimulating locomotor activity in mice generally paralleled analgesic potency (Fig. 2) with the exception of β-endorphin. β-Endorphin even at high doses had little stimulatory effects on locomotion, whereas the enkephalin analogs and opiate alkaloids produced a dose-dependent increase in locomotor activity (Fig. 2). The stimulant actions of enkephalin analogs on locomotor activity were readily antagonized by naloxone (Fig. 2).

Locomotor Activity After Injections of Enkephalin Analogs, β-Endorphin, Morphine and Etorphine

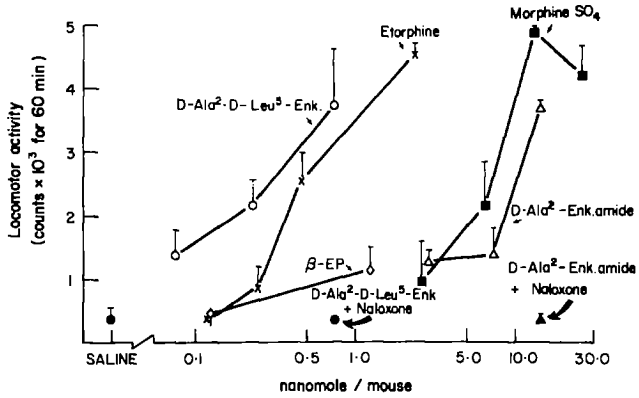

FIG. 2

Three mice were placed in a locomotor activity cage 1 hour before the injection. The locomotor activity was counted for 60 minutes after injection of peptides or opiate alkaloids. Each point represents the mean ± S. E. of 2 to 4 determinations. β-EP; β_c-endorphin, Enk; enkephalin, and Enk. amide; enkephalinamide.

Like β-endorphin (19), D-Ala^2-D-Leu^5-enkephalin also had antinociceptivea activity, but with shorter duration, when it was injected intravenously; relatively large doses, 15 to 20 mg/kg, were required for this effect (Fig. 3).

The qualitative behavioral changes occurring in rats after central administration of opiate alkaloids and peptides have been described in a number of recent publications (8-12,14). In our studies, we find that injections of 50 μg of morphine sulfate into the PAG4 of conscious rats regularly produced, in addition to analgesia and catalepsy, the explosive hyperactivity syndrome (see description by Jacquet and Lajtha, 16). This syndrome was antagonized for approximately 15 minutes by naloxone hydrochloride, but recurred thereafter. Etorphine hydrochloride, 5 μg per rat into the PAG4, produced immediate catalepsy which was completely reversible by naloxone. No hyperactivity was observed with etorphine.

β-Endorphin, 10 μg per rat into the PAG4, and D-Ala ^{2}-D-Leu^5 -enkephalin, 5 μg per rat into the lateral ventricles, produced an average of 11 "wet dog shakes" per animal (9, 20) in the first 5 minutes after injection and then the rats lapses into a cataleptic and analgesic state which was readily reversible by naloxone. The opioid peptides, at doses up to 40 μg per rat, did not trigger the hyperactivity syndrome. The distinctive behavioral effects of the different opiate drugs are summarized in Table 2.

Inhibition of Tail-Flick Response After Intravenous Injection of D-Ala^2 -D-Leu^5 -Enkephalin

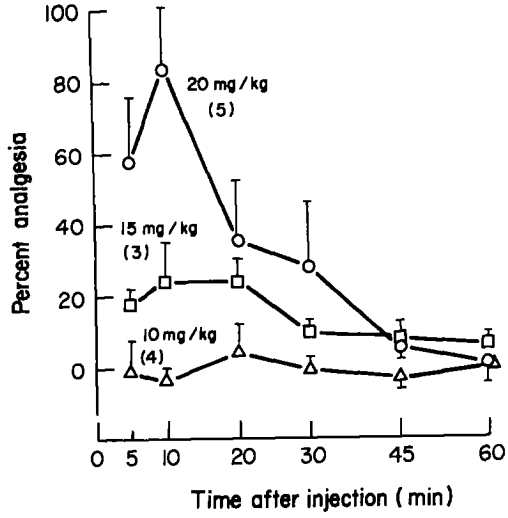

FIG. 3

The degree of analgesia was calculated as $(T_1 - T_0)/(T_2 - T_0) \times 100$, where T_0 is the mean latency of 2 determinations made before drug injections, T_1 is the latencies observed after injections and T_2 is the cutoff time which was set at 10 seconds. The number of mice used are shown in parentheses.

The activity of opiates after central injection is influenced by factors such as lipid solubility of the opiates tested (21), the brain sites where the compounds are injected (22), degradation of the opiate (23) and the affinity characteristics of the opiate for its receptor (24). The results described here show that D-Ala^2 -D-Leu^5 -enkephalin has antinociceptive activity equal to that of β-endorphin. Recently Bajusz et al. (25) have described a synthetic pentapeptide more active than β-endorphin. Resistance to degradation certainly seems to contribute to the potent in vivo activity of the D-Ala^2 - enkephalin analogs but Pert et al. (13) have also suggested that these analogs may bind to an additional site on the opiate receptor. Qualitatively, the principal difference between D-Ala^2 -enkephalin analogs and β-endorphin is the increased activities of the former in stimulating locomotor activity. There appears to be, however, enough qualitative differences among the behavioral effects of opiate

alkaloids and opioid peptides (see Table 2) to support Martin et al.'s (26) and Lord et al.'s (27) suggestions that multiple types of opiate receptors exist in nervous tissue.

TABLE 2

Naloxone-reversible Opiate Effects in Rats*

Drug	Analgesia/Catalepsy		Hyperactivity	Shaking
Morphine Sulfate	+	+	+	0
Etorphine Hydrochloride	+	+	0	0
β-Endorphin	+	+	0	+
Enkephalin Analogs	+	+	0	+

*(+) denotes the presence and (0) denotes the absence of this behavior.

References

1. J. HUGHES, T.W. SMITH, H.W. KOSTERLITZ, L.A. FOTHERGILL, B. A. MORGAN and H.R. MORRIS, Nature 258 577-579 (1975).
2. C.H. LI and D. CHUNG, Proc. Natl. Acad. Sci. USA 73 1145-1148 (1976).
3. A.F. BRADBURY, D.G. SMYTH and C.R. SNELL, Nature 260 793-793 (1976).
4. R. GUILLEMIN, N. LING and R. BURGUS, C.R. Acad. Sci. Series D 282 783-785 (1976).
5. H.H. BUSCHER, R.C. HILL, D. ROMER, F. CARDINAUX, A. CLOSSE, D. HAUSER AND J. PLESS, Nature 261 423-425 (1976).
6. J.D. BELLUZZI, N. GRANT, V. GARSKY, D. SARANTAKIS, C.D. WISE AND L. STEIN, Nature 260 625-626 (1976).
7. H.H. LOH, L.F. TSENG, E. WEI and C.H. LI, Proc. Natl. Acad. Sci. USA 73 2895-2898 (1976).
8. W. FELDBERG and D.G. SMYTH, J. Physiol. 260 30P-31P (1976).
9. F. BLOOM, D. SEGAL, N. LING and R. GUILLEMIN, Science 194 630-632 (1976).
10. Y.F. JACQUET and N. MARKS, Science 194 632-634 (1976).
11. L.F. TSENG, H.H. LOH and C.H. LI, Biochem. Biophys. Res. Commun. 74 390-396 (1977).
12. M. MEGLIO, Y. HOSOBUCHI, H.H. LOH, J.E. ADAMS and C.H. LI, Proc. Natl. Acad. Sci. USA 74 774-776 (1977).
13. C.B. PERT, A. PERT, J.K. CHANG and B.T.W. FONG, Science 194 330-332 (1976).
14. A. PERT, Opiates and Endogenous Opioid Peptides, pp. 87-94, Elsevier/North-Holland Biomedical Press, Amsterdam (1976).
15. E. WEI, S. SIGEL and E.L. WAY, J. Pharmacol. Exp. Ther. 193 56-63 (1975).
16. Y. JACQUET and A. LAJTHA, Science 182 490-492 (1974).
17. J.R. LITCHFIELD, JR. and F. WILCOXON, J. Pharmacol. Exp. Ther.

96 99-113 (1949).
18. C.H. LI, S. LEMAIRE, D. YAMASHIRO and B.A. DONEEN, Biochem. Biophys. Res. Commun. 71 19-25 (1976).
19. L.F. TSENG, H.H. LOH and C.H. LI, Nature 263 239-240 (1976).
20. L. LEYBIN, C. PINSKY, F.S. LaBELLA, V. HAVLICEK and M. REZEK, Nature 264 458-459 (1976).
21. A. HERZ and H. TESCHEMACHER, Adv. Drug Res. 6 79-119 (1971).
22. A. PERT and T. YAKSH, Brain Res. 80 135-140 (1974).
23. J.M. HAMBROOK, B.A. MORGAN, M.J. RANCE and C.F.C. SMITH, Nature 262 782-783 (1976).
24. C.B. PERT and S.H. SNYDER, Science 179 1011-1014 (1973).
25. S. BAJUSZ, A.Z. RONAI, J.I. SZEKELY, L. GRAF, Z.S. DUNAI-KOVACS and I. BERZETEI, FEBS Letters 76 91-92 (1977).
26. W.R. MARTIN, C.G. EADES, J.A. THOMPSON, R.E. HUPPLER and P.E. GILBERT, J. Pharmacol. Exp. Ther. 197 517-532 (1976).
27. J.A.H. LORD, A.A. WATERFIELD, J. HUGHES and H.W. KOSTERLITZ, Opiates and Endogenous Opioid Peptides, pp. 275-280, Elsevier/North Holland Biomedical Press, Amsterdam (1976).