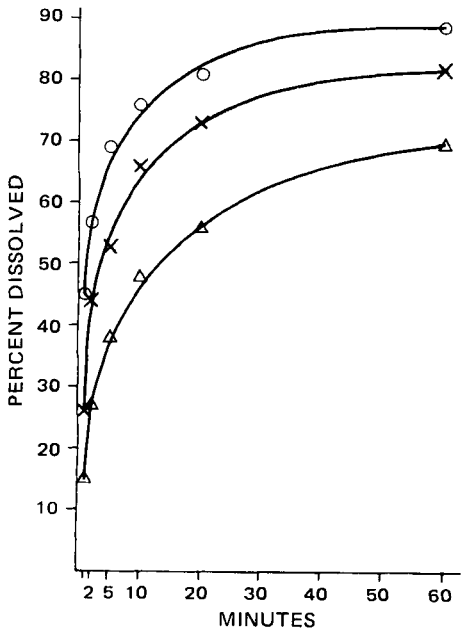

Figure 5—*Dissolution profiles of norethindrone solvent deposited on lactose (particle size less than 150 μm). Key: ○, 2% norethindrone; ×, 10% norethindrone; and △, 20% norethindrone.*

ences when the experiments were carried out in the present beaker apparatus. This result points to the need for caution in interpreting experimental data, because factors such as hydrodynamics and granule size may determine the distinguishability of different formulations.

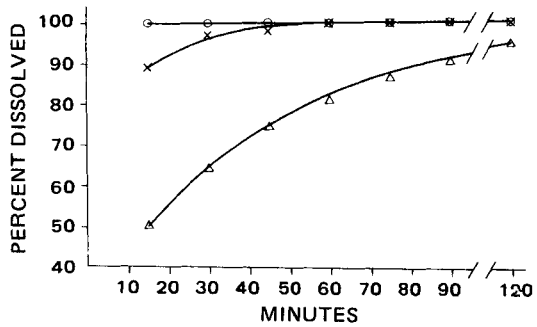

Figure 6—*Dissolution profiles of digoxin solvent deposited on lactose (particle size less than 150 μm). Key: ○, 1% digoxin; ×, 5% digoxin; and △, 25% digoxin.*

REFERENCES

(1) J. B. Termansen, *Arch. Pharm. Chemi*, **66**, 1214 (1959).

(2) D. C. Monkhouse and J. L. Lach, *J. Pharm. Sci.*, **61**, 1430 (1972).

(3) *Ibid.*, **61**, 1435 (1972).

(4) H. Johansen and N. Møller, *Arch. Pharm. Chemi Sci. Ed.*, **4**, 114 (1976).

(5) C. Ampolsuk, J. V. Mauro, A. A. Nyhuis, N. Shah, and C. I. Jarowski, *J. Pharm. Sci.*, **63**, 117 (1974).

(6) N. Shah, R. Pytelewski, H. Eisen, and C. I. Jarowski, *ibid.*, **63**, 339 (1974).

New *In Vivo* Evidence for Narcotic Agonistic Property of Leucine-Enkephalin

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Received February 28, 1977, from the *Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612.* Accepted for publication April 7, 1977.

Abstract □ Administration of leucine-enkephalin or morphine to mice rendered dependent on morphine by pellet implantation inhibited the naloxone-precipitated abstinence syndrome. The withdrawal jumping response was inhibited by morphine or leucine-enkephalin; however, both failed to inhibit withdrawal defecation and rearing behavior. On a molar basis, leucine-enkephalin was half as potent as morphine in inhibiting the abstinence syndrome. New *in vivo* pharmacological evidence for narcotic agonist-like activity of leucine-enkephalin is provided.

Keyphrases □ Leucine-enkephalin—narcotic agonist activity evaluated, mice □ Narcotic activity—leucine-enkephalin evaluated, mice

The discovery of specific opiate receptors in brain and other opiate-sensitive tissues suggested the possible existence of endogenous ligands (1–3). Two pentapeptides, methionine-enkephalin and leucine-enkephalin, were identified (4) and postulated to be endogenous ligands for the opiate receptors in mammalian brain (5–8). Both peptides mimic the ability of morphine to inhibit electrically induced contractions of the guinea pig ileum and mouse vas deferens. These inhibitory effects are antagonized by the opiate antagonist, naloxone (4). Enkephalins also inhibit the stereospecific receptor binding of naloxone in brain homogenate (4). The binding of enkephalins to the opiate receptor is inhibited by a high sodium-ion concentration and enhanced by a high manganese-ion concentration, a response characteristic for opiate agonists (9).

Enkephalins also produce analgesia when administered into the lateral ventricles of rats (10, 11). It has been suggested that enkephalin and morphine receptor sites for analgesia may be similar or identical (12, 13). Recently, methionine-enkephalin was shown to suppress antagonist-induced morphine abstinence in morphine-dependent mice (14). The present report presents new *in vivo* evidence for the similarity of action of morphine and leucine-enkephalin.

EXPERIMENTAL

Male Swiss-Webster mice[1], 25–30 g, were maintained on food and water *ad libitum* in a room maintained on 12-hr light–dark cycles at an ambient temperature of 23 ± 1° and a humidity of 65 ± 2%. Mice were rendered

[1] Scientific Small Laboratories, Arlington Heights, Ill.

morphine dependent by the subcutaneous implantation (15) of a morphine pellet containing 75 mg of morphine base. Three days after implantation, either saline or various doses of leucine-enkephalin[2] in saline were injected intracerebrally (16) in volumes such that each mouse received 0.5 μl/g. Intracerebral injections of India ink were made in 10 mice, and the brain sections revealed that the injection sites were the ventricles.

To precipitate withdrawal, naloxone hydrochloride was injected subcutaneously immediately after the administration of saline or leucine-enkephalin. Three signs of morphine withdrawal—*viz.*, stereotyped jumping behavior, withdrawal defecation, and rearing behavior, were monitored. Stereotyped jumping was used as a quantal response for estimating the median effective dose of naloxone (ED_{50}). After naloxone administration, the mice were placed on a circular platform and the percentage of mice jumping off the platform, the number of fecal boli, and the number of rearings in 15 min were noted for each dose of naloxone.

Eight mice were used for each of the three doses of naloxone to calculate the naloxone ED_{50}, the potency ratio, their 95% confidence limits, and the statistical significance (p values) (17). Similar experiments were conducted with various doses of morphine sulfate substituting for leucine-enkephalin.

RESULTS AND DISCUSSION

Leucine-enkephalin inhibited the naloxone-precipitated abstinence syndrome in morphine-dependent mice. Stereotyped jumping behavior was significantly ($p < 0.05$) inhibited by all doses used. An inverse relationship previously was observed between the degree of dependence and naloxone ED_{50} (18–21). As shown in Table I, leucine-enkephalin increased the naloxone ED_{50} by more than twofold at three different doses (1.75, 3.5, and 7.0 μmoles/kg). With increasing doses of leucine-enkephalin, an apparent increase in the naloxone ED_{50} was noted. However, no significant differences could be seen between the three doses. Earlier investigators (10) also failed to obtain dose–effect data with leucine-enkephalin in determining its analgesic activity.

Leucine-enkephalin was inactive in inhibiting other abstinence signs like withdrawal defecation or rearing behavior. This effect on withdrawal defecation can perhaps be explained by the fact that the drug is active for only 5–10 min when administered intracerebrally, as is evident by its analgesic activity (10). Presumably, it is inactivated rapidly by the proteolytic enzymes in the brain. Also, the solubility of enkephalin may be such that it cannot enter the bloodstream from the brain in sufficient concentrations to affect the intestinal movements.

Administration of morphine also inhibited the withdrawal jumping response. A dose of 0.875 μmole of morphine sulfate/kg increased the naloxone ED_{50}; however, this value was not statistically significant from that for the saline controls. The higher dose of morphine sulfate (1.75 μmoles/kg) significantly inhibited the jumping response, as evidenced by a 4.5-fold increase in naloxone ED_{50} over the saline control. Both doses of morphine sulfate did not produce a significant effect on withdrawal defecation or rearing during the 15-min observation. Comparison of the ED_{50} of naloxone at the same dose (1.75 μmoles/kg) of leucine-enkephalin and morphine showed that morphine sulfate was twice as effective on a molar basis as leucine-enkephalin in inhibiting the morphine abstinence syndrome.

Leucine-enkephalin can inhibit a specific sign of naloxone-precipitated withdrawal in morphine-dependent mice. The withdrawal jumping is a highly characteristic response and is widely used (18–21) for determining the degree of dependence and the intensity of the narcotic abstinence syndrome. In these studies, leucine-enkephalin was half as active as morphine on a molar basis (at 1.75 μmoles/kg) in inhibiting the jumping response. For analgesic activity, morphine was more potent than enkephalin by an order of magnitude (10) or equipotent (11) in the rat. At the doses tested, both leucine-enkephalin and morphine were inactive in producing an inhibitory effect on withdrawal defecation and rearing behavior.

There is strong evidence suggesting morphine and enkephalins behave similarly *in vitro;* however, *in vivo* evidence for similarity is less abundant. The present investigation provides new *in vivo* pharmacological evidence of the narcotic agonistic property of leucine-enkephalin.

[2] Bachem, Inc., Marina Del Rey, Calif.

Table I—Comparison of the Effects of Leucine-Enkephalin and Morphine Sulfate, Administered Intracerebrally, on the Naloxone ED_{50} in Morphine-Dependent Mice[a]

Treatment	Dose, μmoles/kg	Naloxone ED_{50}, μg/kg	Potency Ratio[b]
Saline	—	166 (109–255)	—
Morphine sulfate	0.875	200 (117–341)	1.20 (0.62–2.34)
	1.75	729 (476–1117)	4.39 (2.47–7.81)[c]
Leucine-enkephalin	1.75	366 (239–561)	2.20 (1.20–3.96)[c]
	3.50	387 (242–620)	2.33 (1.23–4.31)[c]
	7.00	409 (261–643)	2.46 (1.37–4.43)[c]

[a] The figures in parentheses represent the 95% confidence limits. [b] Potency ratios were calculated according to Ref. 17 for comparison of ED_{50} values in drug-treated groups with the saline control. [c] $p < 0.05$ *versus* saline controls.

REFERENCES

(1) A. Goldstein, L. I. Lowney, and B. K. Pal, *Proc. Natl. Acad. Sci. USA,* **68,** 1742 (1971).

(2) E. J. Simon, J. M. Hiller, and I. Edelman, *ibid.,* **70,** 1947 (1973).

(3) C. B. Pert and S. H. Snyder, *ibid.,* **70,** 2243 (1973).

(4) J. Hughes, T. W. Smith, H. W. Kosterlitz, L. A. Fothergill, B. A. Morgan, and H. R. Morris, *Nature,* **258,** 577 (1975).

(5) L. Terenius and A. Wahlstrom, *Acta Pharmacol. Toxicol.,* **35,** 55 (1974).

(6) J. Hughes, *Brain Res.,* **88,** 295 (1975).

(7) G. W. Pasternak, R. Goodman, and S. H. Snyder, *Life Sci.,* **16,** 1765 (1975).

(8) H. Teschemacher, K. E. Opheim, B. M. Cox, and A. Goldstein, *ibid.,* **16,** 1771 (1975).

(9) R. Simantov and S. H. Snyder, *ibid.,* **18,** 781 (1976).

(10) J. D. Belluzzi, N. Grant, V. Garsky, D. Sarantakis, C. D. Wise, and L. Stein, *Nature,* **260,** 625 (1976).

(11) J. B. Malick and J. M. Goldstein, *Pharmacologist,* **18,** 120 (1976).

(12) H. W. Kosterlitz and J. Hughes, *Life Sci.,* **17,** 91 (1975).

(13) H. Akil and J. C. Liebeskind, *Brain Res.,* **94,** 279 (1975).

(14) H. N. Bhargava, *Eur. J. Pharmacol.,* **41,** 81 (1977).

(15) *Ibid.,* **36,** 259 (1976).

(16) T. J. Haley and W. G. McCormick, *Br. J. Pharmacol.,* **12,** 12 (1957).

(17) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.,* **96,** 99 (1949).

(18) E. L. Way, H. H. Loh, and F. H. Shen, *ibid.,* **167,** 1 (1969).

(19) Y. Maruyama and A. E. Takemori, *ibid.,* **185,** 602 (1973).

(20) D. L. Francis and F. Schneider, *Br. J. Pharmacol.,* **41,** 424p (1971).

(21) H. N. Bhargava, *Psychopharmacology,* **52,** 55 (1977).

ACKNOWLEDGMENTS

The author thanks Dr. Norman R. Farnsworth for his interest, Mr. George Matwyshyn for technical assistance, and Ms. Dorothy Wilson for help in the preparation of this manuscript.