Analgesic activity of the naturally occurring heptapeptide
[Met]enkephalin-Arg⁶-Phe⁷

Charles E. Inturrisi*, Jason G. Umans*, Douglas Wolff*, Alvin S. Stern†, Randolph V. Lewis†, Stanley Stein†, and Sidney Udenfriend†

*Department of Pharmacology, Cornell University Medical College, New York, New York 10021; and †Roche Institute of Molecular Biology, Nutley, New Jersey 07110

Contributed by Sidney Udenfriend, May 20, 1980

ABSTRACT [Met]Enkephalin-Arg⁶-Phe⁷ is an opiate-like peptide normally found in the adrenal gland and brain that has analgesic (antinociceptive) activity when administered directly into the cerebral ventricles of mice. On a molar basis, [Met]-enkephalin-Arg⁶-Phe⁷, with a median effective dose (ED₅₀) of 38.5 nmol/mouse, is 8 times more potent than [Met]enkephalin. As with [Met]enkephalin, analgesic activity is blocked by naloxone and intravenous administration does not produce characteristic opiate effects in tests for analgesic, anti-diuretic, or anti-diarreal activity. These findings suggest that [Met]enkephalin-Arg⁶-Phe⁷ may be at least as important as the enkephalins in the postulated enkephalin system mediating pain and analgesia.

The pharmacologic characterization of the opiate-like pentapeptides [Met]enkephalin and [Leu]enkephalin revealed that intracerebroventricular (1, 2) or spinal subarachnoid (3) administration can produce a behaviorally defined analgesia in response to nociceptive stimuli. The opiate actions of these peptides suggest that they may be endogenous ligands for the opiate receptor. The distribution of enkephalin-containing neurons, the transient analgesic action of [Met]enkephalin and [Leu]enkephalin in laboratory animals, and reports of enkephalin-like material in human cerebrospinal fluid during stimulation produced analgesia have led some workers to propose that the enkephalins are involved in brain systems modulating the perception of pain (4, 5). Although the enkephalins were first isolated from mammalian brain (6), they are now known to be present in other tissues, including intestine and adrenal medulla (7). Recent studies of the biosynthetic pathway of the enkephalins by Stern et al. (7) led to the isolation from adrenal chromaffin granules of several novel enkephalin-containing peptides and proteins. One of these, the heptapeptide [Met]enkephalin-Arg⁶-Phe⁷, which is also present in beef striatum, human putamen, and human globus pallidus (8), has been chemically synthesized in amounts sufficient for pharmacological characterization.

MATERIALS AND METHODS

Male Swiss Webster mice (20–30 g) and male Sprague-Dawley rats (200–250 g), purchased from Taconic Farms (Germantown, NY), were used in these experiments. [Met]Enkephalin was purchased from Boehringer Mannheim, camel β-endorphin (β₈-endorphin) from Peninsula Laboratories (San Carlos, CA), and morphine sulfate from Mallinkrodt. Naloxone-HCl was a gift from Endo Laboratories (Garden City, NY). Prostaglandin E₂ was a gift from Upjohn. [Met]Enkephalin-Arg⁶-Phe⁷ was synthesized by Peninsula Laboratories and purified by us by high-performance liquid chromatography (7).

Drug Administration. Intracerebroventricular (i.c.v.) injections were made by a modification of Pedigo et al. (9) of the method of Hallay and McCormick (10). Intravenous (i.v.) injections were made into a lateral tail vein. Rats were prepared for the i.v. infusion of drug by cannulation of the right external jugular vein.

Analgesic (Antinociceptive) Assay. Analgesic activity after i.c.v. administration of opioid was measured by the radiant-heat tail-flick procedure of D’Amour and Smith (11) as modified by Dewey et al. (12). A control latency of 2–4 sec and a cutoff time of 10 sec were used. The response was calculated as the percentage of maximal response by the following formula (13): [(test − control)/(10 − control)] X 100 = % maximal possible effect. Dose–response curves were constructed by plotting the percent maximal possible effect against the logarithm of the dose on probit paper. The median effective dose (ED₅₀), slope, and relative potency ratios with their 95% confidence limits were calculated by the method of Litchfield and Wilcoxon (14). At least eight mice were tested at each dose with three or four dose levels used to determine each ED₅₀.

Antidiuretic Assay. Antidiuretic activity after i.v. administration of opioid was assessed by the suppression of prostaglandin E₂-induced diuresis, by the method of Sanner (15).

Antidiuretic Assay. Antidiuretic activity after i.v. administration of opioid was assessed by the prolongation of the excretion of intraperitoneally administered water, by the method of Inturrisi and Fujimoto (16).

RESULTS AND DISCUSSION

The analgesic dose–response curves after i.c.v. administration of the compounds of interest are given in Fig. 1. Each produced a dose-related inhibition of the tail-flick response of mice. The effects of [Met]enkephalin-Arg⁶-Phe⁷ and [Met]enkephalin peaked at 2–4 min and were dissipated by 10 min. The analgesic effect of morphine and β₈-endorphin reached a peak later (10–15 min) and lasted substantially longer (60 min). Analysis of the data revealed no significant deviation from parallelism; the ED₅₀ and relative potency estimates are presented in Table 1. On a molar basis, [Met]enkephalin-Arg⁶-Phe⁷ was approxi-
mately 8 times more active than [Met]enkephalin but 1/63rd as potent as morphine and 1/1156th as potent as β-endorphin. In vivo estimates of relative potency are the result of receptor interactions and pharmacokinetic factors. Thus, it is not surprising that the more labile pentapeptides and presumably more labile [Met]enkephalin-Arg⁶-Phe⁷ have a shorter duration of action and are less potent when compared to morphine or β-endorphin (17).

Pretreatment with naloxone, an opiate antagonist, diminished or prevented the response to approximately equianalgesic doses of [Met]enkephalin-Arg⁶-Phe⁷ and morphine (Table 2). Pretreatment with naloxone or saline followed by i.c.v. administration of saline was without effect (not shown in Table 2). A larger dose of naloxone was required to block the analgesic response to [Met]enkephalin-Arg⁶-Phe⁷ compared to morphine. This suggests that different receptors or binding affinities may be involved.

Because [Met]enkephalin-Arg⁶-Phe⁷ is present in the chromaffin granules of the adrenal medulla and stimulation of the adrenal causes release of enkephalins (8) and their precursors, a consideration of the systemic action seemed appropriate. We found that neither [Met]enkephalin-Arg⁶-Phe⁷ nor [Met]enkephalin produced characteristic opiate effects after systemic administration. Doses of the heptapeptide up to 11.4 μmol/kg (10 mg/kg) did not produce analgesic, antidiuretic, or antidiarrheal effects in mice whereas equimolar doses of morphine produced a significant effect in each of these opiate tests. These results are consistent with the report of Dairman et al. (18) that in mice [Met]enkephalin has little or no systemic antidiarrheal activity whereas β-endorphin is active. In rats the i.v. infusion of 0.10 mg of [Met]enkephalin-Arg⁶-Phe⁷ per min for 30 min did not produce analgesia or gross behavioral changes. Undoubtedly, the absence of effects is due predominantly to rapid degradation of [Met]enkephalin-Arg⁶-Phe⁷ by blood and tissue as is the case with the enkephalins.

[Met]Enkephalin-Arg⁶-Phe⁷ is present in human putamen and beef striatum in amounts comparable to that of [Leu]enkephalin and about one-fourth that of [Met]enkephalin (8). Although [Met]enkephalin-Arg⁶-Phe⁷ can be converted in vitro to [Met]enkephalin by digestion with trypsin and carboxypeptidase B, the greater analgesic potency of the heptapeptide in vivo argues against its action being merely that of a metabolic intermediate. Thus, in the radioreceptor binding assay using neuroblastoma-glioma hybrid cells with [³H]tyrosine-labeled [Leu]enkephalin as the competing ligand, the concentration of [Met]enkephalin and [Leu]enkephalin necessary for 50% inhibition is 3.5 nM and that of [Met]enkephalin-Arg⁶-Phe⁷ is 6.8 nM (8). In addition to [Met]enkephalin-Arg⁶-Phe⁷, several opioid hexapeptides and another heptapeptide have been isolated from the adrenal medulla and demonstrated in brain as well (8).

Whatever their physiologic roles, [Met]enkephalin-Arg⁶-Phe⁷ and the other opioid heptapeptides and hexapeptides contribute to the mapping of the enkephalin pathway in the central ner-

---

### Table 1. Analgesic activity after i.c.v. administration

<table>
<thead>
<tr>
<th>Drug</th>
<th>Time of peak effect, min</th>
<th>ED₅₀⁺⁻</th>
<th>Relative potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td>20</td>
<td>0.63 (0.29–0.87)</td>
<td>1</td>
</tr>
<tr>
<td>[Met]Enkephalin-Arg⁶- Phe⁷</td>
<td>2–4</td>
<td>38.5 (23.7–62.3)</td>
<td>0.016</td>
</tr>
<tr>
<td>[Met]Enkephalin-β-Endorphin</td>
<td>4</td>
<td>330.0 (248–459)</td>
<td>0.0019</td>
</tr>
</tbody>
</table>

* 95% confidence limits are given in parentheses.

---

### Table 2. Naloxone blockade of analgesia

<table>
<thead>
<tr>
<th>Pretreatment*</th>
<th>Analgesic tested†</th>
<th>% MPE†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>Morphine, 0.87 nmol</td>
<td>61</td>
</tr>
<tr>
<td>Naloxone, 2 mg/kg</td>
<td>Morphine, 0.87 nmol</td>
<td>0</td>
</tr>
<tr>
<td>Saline</td>
<td>[Met]Enkephalin-Arg⁶-Phe⁷, 57.4 nmol</td>
<td>72</td>
</tr>
<tr>
<td>Naloxone, 2 mg/kg</td>
<td>[Met]Enkephalin-Arg⁶-Phe⁷, 57.4 nmol</td>
<td>27</td>
</tr>
<tr>
<td>Naloxone, 4 mg/kg</td>
<td>[Met]Enkephalin-Arg⁶-Phe⁷, 57.4 nmol</td>
<td>5</td>
</tr>
</tbody>
</table>

* Naloxone was administered subcutaneously.
† Doses are expressed as nmol/mouse i.c.v.
‡ MPE, maximal possible effect.
vous system which is carried out by immunohistochemical procedures directed toward the pentapeptide sequence.

This work was supported in part by National Institute of Drug Abuse Grants DA-01457 and GM-26145. J.G.U. is the recipient of an Insurance Medical Scientist Scholarship Fund award from the Prudential Insurance Company.