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# Analgesia After Peripheral Administration of Enkephalin and Endorphin Analogues

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KASTIN, A. J., M. T. JEMISON AND D. H. COY. *Analgesia after peripheral administration of enkephalin and endorphin analogues*. PHARMAC. BIOCHEM. BEHAV. 11(6) 713–716, 1979.—Several analogues of Met-enkephalin and β-endorphin were tested for their analgesic properties after systemic injection. The latencies of mice to flick their tails away from a source of heat revealed that analogues of the opiate peptides can cause analgesia when injected by this route. In particular, compounds specifically designed to be more lipophilic or to possess additional binding sites were shown to be potent analgesic agents after peripheral administration.

Analgesia β-Endorphin Met-enkephalin Enkephalin Endorphin Lipophilic Binding sites

SEVERAL analogues of Met-enkephalin and β-endorphin were tested for their analgesic properties after systemic injection. The latencies of mice to flick their tails away from a source of heat revealed that analogues of the opiate peptides can cause analgesia when injected by this route. In particular, compounds specifically designed to be more lipophilic or to possess additional binding sites were shown to be potent analgesic agents after peripheral administration.

Use of the peripheral route of administration facilitated our demonstration in 1976 that the behavioral effects of the opiate peptides can be dissociated from their direct opiate actions [7,10]. Similar behavioral effects of other brain peptides had also been shown after peripheral injection [6]. Administration by this route rather than centrally facilitates animal experimentation and makes possible eventual clinical applications. Several analogues of Met-enkephalin and β-endorphin were tested for analgesia by the "tail-flick" method after peripheral injection.

## METHOD

### *Animals*

Male, albino, ICR, mice weighing 15–17 g on arrival were purchased from Gibco, Inc., Madison, WI. They were kept on a 12 hr light: 12 hr dark cycle in a sound-attenuated room and fed ad lib for two days before experimentation.

### *Apparatus*

The mouse was placed in a 2.5 cm I.D. Plexiglas tube 5 cm long during the entire period of testing. The mouse's tail was set in a groove so that its middle portion lay over a 0.3×3.0 cm slit situated 1.0 cm above the rheostat-modulated 100 W high-intensity GE bulb which served as the source of radiant heat. In position, the tail prevented a photo-transistor from being energized by the optic source. As the temperature increased, the mouse flicked its tail away from the lamp, allowing the 9 V optic relay circuit to be completed. This opened a 110 V line which stopped the lamp and timer.

### *Procedure*

Mice were tested before injection and 15, 30, 45, 60, 90, 120, 150, 180, 240, and 300 min afterwards. A test consisted of 4 trials, separated by 1 to 1.5 min. Each trial measured the time required for a mouse to flick its tail away from the source of heat. Only the mean of the last two trials was used in each test period. To prevent damage to the tail, no trial was allowed to continue for more than 7 sec. During the preinjection control trial, the temperature was adjusted so that more than 90% of the mice removed their tails 2–5 sec after onset of the heat, a criterion for injection of the test material. If analgesia persisted throughout the test period, naloxone (Endo Labs) was administered (4 mg/kg) to ascertain that the tail was not so damaged as to prevent the heat from being felt.

### *Peptides*

All peptides were synthesized by solid phase methods [2]. They were dissolved daily in 30% propylene glycol made in 0.9% NaCl acidified to 0.01 M with acetic acid. The solutions, including diluent, were coded and injected intraperitoneally (IP) in a volume between 0.4–0.6 ml, depending upon the weight of the individual mouse.

### *Analysis of Data*

The percent analgesia at each time after injection was calculated by the following formula: (test latency—control

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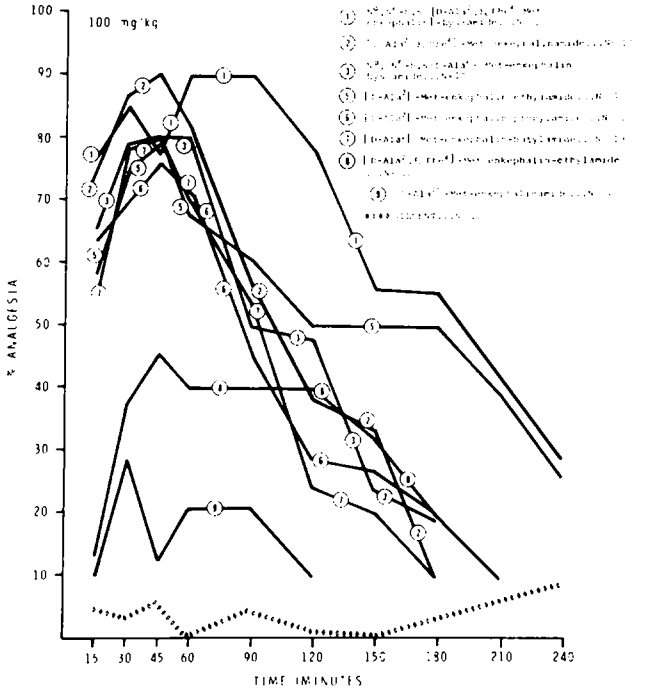


FIG. 1. Percent analgesia after IP injection of enkephalin analogues (100 mg/kg).

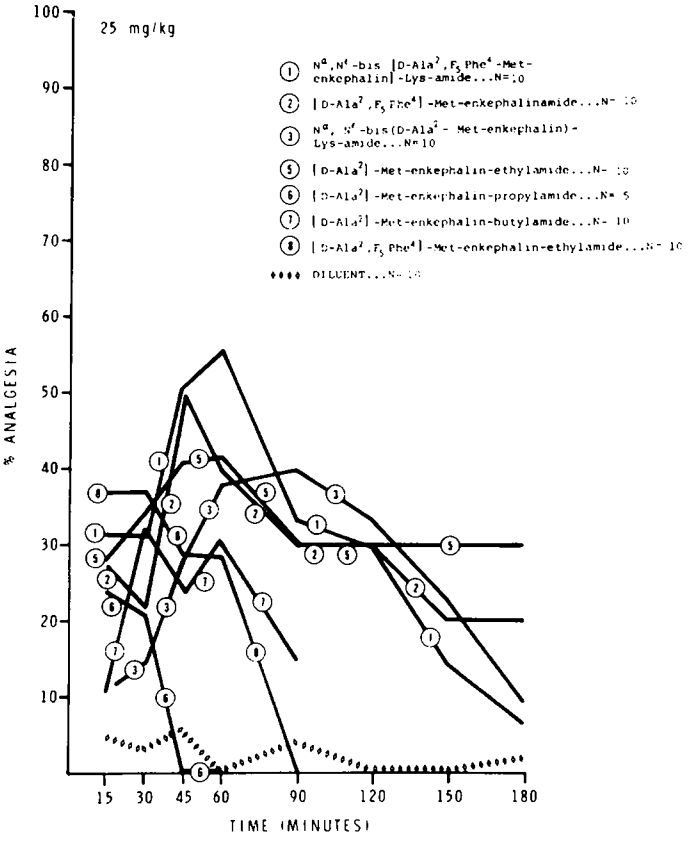


FIG. 2. Percent analgesia after IP injection of enkephalin analogues (25 mg/kg).

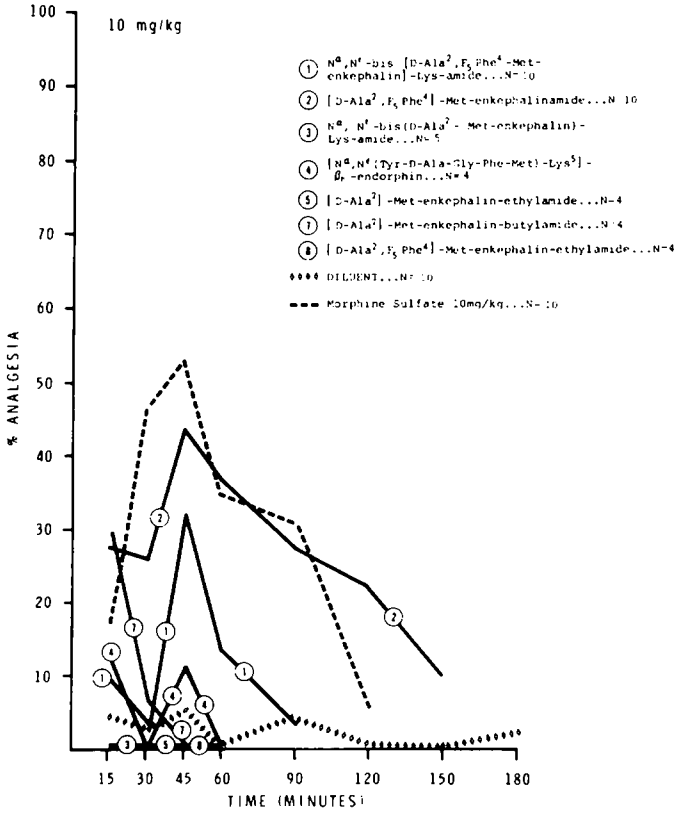


FIG. 3. Percent analgesia after IP injection of enkephalin and endorphin analogues (10 mg/kg).

latency/7 sec—control latency) × 100. At doses of 100 mg/kg body weight and 25 mg/kg, the data obtained for the enkephalin analogues were analyzed separately from those obtained for the endorphin analogues according to dose. The data for both groups of opiate peptides obtained at 10 mg/kg were analyzed together, as were those for the dose of 1 mg/kg. Analysis of variance was used in each case, at the time of maximal analgesia, followed by Duncan's Multiple Range Test. Relative potencies were determined by the area under the curve from 15 to 180 min measured by a Dietzgen compensating polar planimeter (D1803D).

## RESULTS

At the high dose of 100 mg/kg IP, Met-enkephalin exerted no reliable analgesic effect, as expected. Even (D-Ala²)-Met-enkephalinamide, many times more potent than Met-enkephalin *in vitro* [3] and in the tail-flick test after intraventricular administration [9,11], caused no statistically significant increase in analgesia when injected IP at this dose. However, the pentafluorophenylalanine analogue and its dimer were very active at 100 mg/kg. The ethylamide form of (D-Ala²)-Met-enkephalinamide, like the pentafluorinated dimer, also had a long duration of action. This effect was completely blocked by pretreatment with naloxone. The analogue representing a combination of the pentafluorinated and ethylamide derivatives, surprisingly, was only about half as potent as either of the single derivatives, although it was more potent than (D-Ala²)-Met-enkephalinamide. All ana-

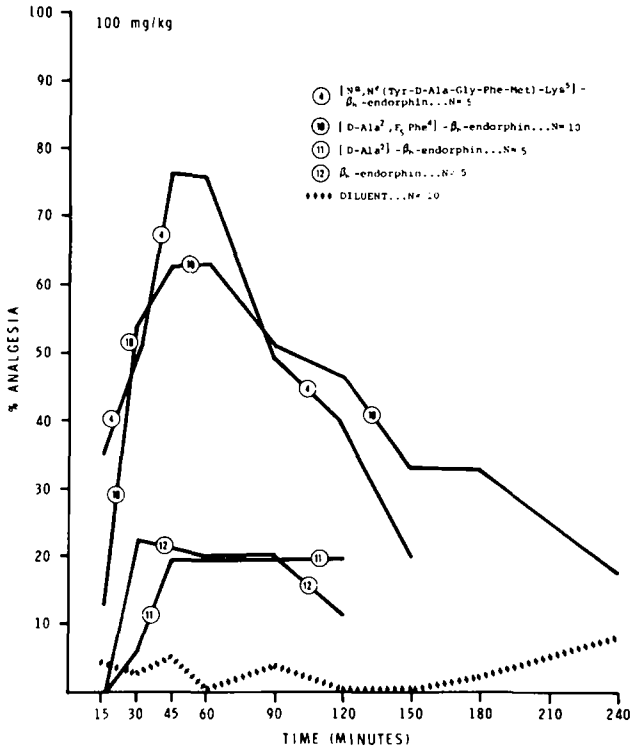


FIG. 4. Percent analgesia after IP injection of endorphin analogues (100 mg/kg).

logues (Fig. 1) tested at this dose (100 mg/kg) caused significantly ($p<0.01$) more analgesia than diluent, Met-enkephalin, and (D-Ala$^2$)-Met-enkephalinamide, except the pentafluorinated ethylamide derivative which was only statistically greater than diluent.

At an IP dose four times less (25 mg/kg), the most analgesia was again seen after injection of the pentafluorinated dimer (Fig. 2). At its peak, this was significantly ($p<0.05$) greater than diluent. The times of maximal analgesia appeared to vary more from the usual peak time of 45 min at the smaller doses.

At the reduced dose of 10 mg/kg IP a pentafluoro-derivative of enkephalin was still the most potent and significantly greater than diluent (Fig. 3). No significant difference was found between the (D-Ala$^2$, F$_5$Phe$^4$)-Met-enkephalinamide and morphine sulfate administered IP at the same dose. It is not clear why the monomer form of the pentafluorophenylalanine analogue was more potent at 10 mg/kg than the dimer, in contrast to the findings at 25 mg/kg and 100 mg/kg. However, this difference was not statistically significant and may reflect the large variability found in the assay.

With the (D-Ala$^2$)-derivative of $\beta_h$-endorphin, as with the (D-Ala$^2$)-derivative of Met-enkephalin, increased activity had been found in vitro [1] as well as after intraventricular administration in the tail flick test [12]. Yet even at the high dose of 100 mg/kg IP, the analgesia caused by (D-Ala$^2$)-$\beta_h$-endorphin was not significantly different from that observed after IP injection of $\beta_h$-endorphin or diluent (Fig. 4), despite its increased resistance to enzymatic degradation [5]. The analgesia caused by the pentafluorophenylalanine and dimer analogues of (D-Ala$^2$)-$\beta_h$-endorphin was statistically different from diluent at 100 mg/kg. At a dose of

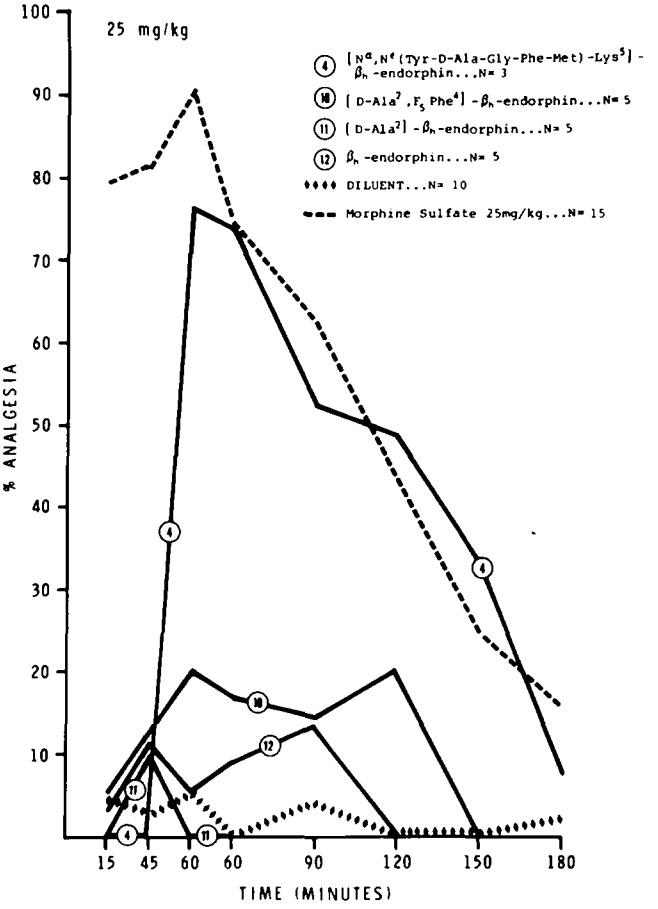


FIG. 5. Percent analgesia after IP injection of endorphin analogues (25 mg/kg).

25 mg/kg IP, the results observed after administration of the dimer of (D-Ala$^2$)-$\beta_h$-endorphin were significantly ($p<0.01$) greater than those after diluent or any of the endorphins tested and statistically the same as morphine, but the pentafluoro-derivative was not active (Fig. 5).

## DISCUSSION

The results indicate that several types of modifications of the opiate peptides produce analogues capable of causing analgesia when administered peripherally. This contrasts with the negligible analgesic activity of the parent compounds, Met-enkephalin and $\beta$-endorphin, after systemic injection.

Lipophilicity is an important consideration in devising potent analogues, particularly if they are to be injected peripherally [4,6]. The replacement of phenylalanine with pentafluorophenylalanine should greatly increase lipophilicity without substantially modifying the conformation of the molecule. Similar considerations should apply to the increased lipophilicity expected after addition of ethyl-, propyl-, and butyl-alkyl groups to C-terminal amides. Although these alkyl and pentafluoro additions were found to increase analgesia in most cases, this was not a uniform occurrence (e.g. the pentafluoronated enkephalin ethylamide at the smaller doses). Moreover, other considerations con-

tribute to the increased potency of a pentafluoro-analogue since it is also more potent after intraventricular injection [4] and is more resistant than the Sandoz analogue FK 33-824 to degradation by brain enzymes in vitro [8].

Increased potency would also be expected for analogues in which the ability of each molecule to bind to more than one receptor is increased. It was found that the dimers of both (D-Ala$^2$)-Met-enkephalinamide and (D-Ala$^2$)-$\beta_h$-endorphin were more potent than the corresponding monomeric analogues or the parent compounds. The stabilizing effect of (D-Ala$^2$)-substitutions on the $\beta$-bend as well as increased resistance to degradation have been discussed elsewhere [3, 5, 8, 11].

The high activities after peripheral administration of (D-Ala$^2$, $F_5$Phe$^4$)-$\beta_h$-endorphin and the (D-Ala$^2$)-dimer analogue confirm that the $\beta_h$-endorphin chain can be modified quite readily to facilitate analgesia. On a molar basis, these two large peptides were the most potent compounds examined in this assay system and compare very favorably with morphine.

No significant analgesia was observed with any of the enkephalin or endorphin analogues at the dose of 1 mg/kg IP. The behavioral effects, however, which we have been observing since 1976 after systemic administration of some of these analogues and their parent compounds did not require doses higher than this [6]. It would seem, then, that some pronounced brain effects may be dissociated from the analgesic effects.

In general, the potency of the enkephalin and endorphin analogues in inducing analgesia tended to correspond to their potency in binding to opiate receptors and inhibition of electrically induced contractions in the *vas deferens*. Preliminary data indicate, however, that there may be more exceptions to this generality than we have discussed [4] previously. Regardless, the results demonstrate that analgesia can be induced by several analogues of the opiate peptides after peripheral administration.

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