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MIF-I's Differential Actions as an Opiate Antagonist

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KASTIN, A. J., R. D. OLSON, R. H. EHRENSING, M. C. BERZAS, A. V. SCHALLY AND D. H. COY. *MIF-I's differential actions as an opiate antagonist.* PHARMAC. BIOCHEM. BEHAV. 11(6) 721–723, 1979.—The effects of MIF-I (Pro-Leu-Gly-NH_2) were examined in three experimental conditions in which the opiate antagonist naloxone is active. MIF-I was found to block the analgesic effects of enkephalins and also morphine in the tail-flick test but not in the *vas deferens* assay. Unlike naloxone, MIF-I did not seem to reduce food intake in VMH-lesioned rats. The results suggest the possibility that MIF-I may represent a class of naturally occurring opiate antagonists with varying activities in independent situations.

Peptide Opiate Receptor MIF-I Analgesia Food intake Vas deferens

MIF-I (Pro-Leu-Gly-NH_2) is the hypothalamic tripeptide which was used in the first demonstration of the direct CNS effects of a brain peptide not involving the pituitary gland [5,9]. It has subsequently been proposed and demonstrated that even the brain opiates, enkephalin and endorphin, may generate multiple independent actions [5,6]. This led us to formulate the concept of the dissociation of narcotic and behavioral actions of the opiate peptides to account for data demonstrating differential actions of the same peptide in independent test systems [5,6]. MIF-I has been reported to affect opiate actions in several tests, often interpreted to indicate opposing actions of MIF-I [10,11]. This preliminary report will indicate that MIF-I has antagonistic opiate actions resembling those of naloxone in one condition but apparently has no effect in other conditions in which naloxone is highly active.

METHOD

Tail-Flick Assay

The tail of a male, albino mouse was placed over a source of radiant heat and the time required for movement of the tail was recorded as described elsewhere [4]. An upper limit of seven seconds was set so as to prevent damage to the tail and was used as the criterion for analgesia at 30 min. All injections were made intraperitoneally (IP). The assays were done in six sets. In the first set, 12 mice received synthetic MIF-I (10 mg/kg, IP) followed 10 min later by (D-Ala^2)-Met-enkephalin-ethylamide (100 mg/kg, IP). Twelve different mice received diluent (30% propylene glycol in saline acidified to pH 3.5 with acetic acid) followed by the same enkephalin analogue. A second set of 32 mice was treated in a manner identical to the first set except that morphine sulfate (10 mg/kg, IP) without propylene glycol was used in place of the opiate peptide. In a third set (D-Ala^2, F_5Phe^4)-Met-enkephalinamide (50 mg/kg, IP) was the opiate used. In a fourth set, a smaller dose of MIF-I (5 mg/kg, IP) was used with the morphine (10 mg/kg, IP). The next two sets were identical to the second except that in the fifth set morphine (10 mg/kg, IP) and MIF-I (10 mg/kg, IP) or diluent were given at the same time and in the sixth set the morphine was given ten minutes before the MIF-I or diluent.

Vas Deferens Assay

A *vas deferens* obtained from a male, albino mouse was suspended in a 5 ml bath maintained at 37°C. Electrically stimulated contractions were recorded by an isometric force displacement transducer on a polygraph as previously described [3].

VMH Food Intake

Food intake after injection of MIF-I (5 mg/kg, IP) was compared in two groups. In one group of three rats, bilateral VMH-lesions had produced marked obesity. Another group consisted of three unoperated controls receiving 5 mg/kg MIF-I and five intact rats receiving 10 mg/kg MIF-I. Baseline food intake was determined one and four hours after injection of diluent. The next day, intake was measured one and four hours after MIF-I. Histological examination of 50 μm coronal sections stained with cresyl violet confirmed the extent of the desired lesion. Percent difference from baseline was determined for each rat as described elsewhere [7].

RESULTS

Tail Flick Assay

Only two of 12 mice receiving (D-Ala^2)-Met-enkephalin-ethylamide showed analgesia when pretreated with MIF-I

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(10 mg/kg, IP) in contrast to seven of 12 mice pretreated with diluent. The difference was statistically significant ($p<0.05$) by the Fisher Exact Probability Test. More consistent results were seen with morphine. As with the enkephalin analogue, the analgesia was an "all-or-none" phenomenon, but a higher percentage of animals showed it. Whereas 15/16 mice receiving diluent and morphine showed maximal analgesia, 1/16 mice receiving 10 mg/kg MIF-I ($p<0.01$) and 0/10 mice receiving 5 mg/kg MIF-I before the morphine showed analgesia ($p<0.01$). Only 1/16 mice receiving MIF-I (10 mg/kg, IP) at the same time as the morphine and 2/9 mice receiving MIF-I ten minutes later showed analgesia in contrast to 5/6 of them given diluent simultaneously ($p<0.01$) and 4/4 given diluent after ($p<0.05$) the morphine. However, MIF-I was more effective in blocking the analgesia induced by morphine when it was given ten minutes before the morphine than when it was given simultaneously or afterwards. J. Dunn and V. B. Ciofalo have made somewhat similar observations (personal communication). A tendency in the same direction was seen with the use of (D-Ala^2, F$_5$Phe^4)-Met-enkephalinamide; one of the five mice pretreated with MIF-I (10 mg/kg) showed analgesia in contrast to six of nine receiving diluent before the morphine. MIF-I (10 mg/kg) alone had no analgesic actions in the three mice tested.

Vas Deferens Assay

MIF-I, at concentrations ranging from 10 ^{9}M to 10^{-5}M, had no effect in antagonizing the potent enkephalin analogue (D-Ala^2, F$_5$Phe^4)-Met-enkephalinamide, in this system. The contractions were not affected by MIF-I added one minute before the enkephalin analogue or at the same time, in contrast to the marked antagonism caused by naloxone. MIF-I was also ineffective in antagonizing the effects of somatostatin (9×10^{-8}M). By itself, MIF-I did not alter the electrically stimulated contractions.

VMH Food Intake

One hour after injection of MIF-I (5 mg/kg) control rats ate the same amount (100%) of food as they had on the previous day (after injection of diluent) and rats with VMH-lesions consumed 86% of the amount eaten during the baseline period. Four hours after injection of MIF-I (5 mg/kg) rats with VMH lesions consumed 103% of that consumed after diluent, and the control rats ate 104%. Intact rats receiving 10 mg/kg MIF-I ate 111% baseline after one hour and 110% after four hours. None of these changes were statistically significant.

DISCUSSION

This preliminary communication reports the effects of MIF-I in three experimental conditions in which naloxone has been found to show marked effects [3, 4, 7]. First, in the tail flick test of analgesia, MIF-I was found to exert effects similar to naloxone. Both compounds acutely reversed the analgesia induced by morphine and also that induced by an enkephalin. Second, in the *vas deferens* assay, another classical method for determining opiate actions, MIF-I was without effect. Naloxone, by contrast, reverses the inhibition caused by the opiate peptides. Third, in the VMH-lesioned rats, naloxone has been found to markedly reduce food intake, as it does in unoperated rats [7]. MIF-I was ineffective under the experimental conditions used.

These results indicate that MIF-I duplicated the effects of the classical opiate antagonist naloxone in its ability to block analgesic effects but seemed not to do so in other situations where naloxone is active. The early work in this area concentrated on facilitation of opiate tolerance by MIF-I [10] without commenting upon the possibility of MIF-I representing a class of naturally occurring antagonists. Studies of the effects of MIF-I in opiate dependence by Walter *et al.* [11] showed conflicting results to those reported by Van Ree and deWied [10]. The blocking [11] rather than facilitation [10] of physical opiate dependence by MIF-I directed our attention to the naloxone-like properties of MIF-I.

The possibility arises that MIF-I could represent a class of naturally occurring opiate antagonists which may correspond to naloxone in a manner similar to that in which the opiate peptides parallel plant alkaloids. Just as we have observed that various peptides show different profiles in neuropharmacological tests [5,9], and that the narcotic and behavioral effects of brain opiates can be differentiated [5,6], it is expected that several natural peptides will be found to mimic the actions of naloxone at the varying types of opiate receptors [1,8] for which widespread evidence is accumulating. The greater variability in obtaining analgesia after opiate peptides compared to morphine might reflect these differences in receptors and their sensitivity, although the effects of varying metabolism cannot be ignored. A recent report, however, failed to find an effect of MIF-I on pain even though MIF-I did reduce morphine-induced catalepsy [2].

The eventual description of specific antagonists which may also act on different populations of receptors, different states of the same receptors, or on non-opiate as well as opiate receptors may necessitate a more flexible concept of this issue. Secondary actions on other endogenous opiate antagonists, effects on the metabolism or distribution of natural opiates, and the differential location of opiate receptors in the periphery as well as brain must also be considered. Regardless, it is clear that under the conditions tested MIF-I does not act in a manner identical to naloxone, but can antagonize opiate actions in at least one situation while showing no activity in others.

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