Pharmacology Biochemistry & Behavior, Vol. 21, pp. 975–978, 1984. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

BRIEF COMMUNICATION

Antagonism of Morphine Analgesia by Prolyl-Leucyl-Glycinamide (MIF-1) In Humans[1]

RUDOLPH H. EHRENSING, ABBA J. KASTIN* AND GARY F. MICHELL

Bintliff-Johnson Research Laboratory, Department of Psychiatry
Ochsner Clinic and Alton Oschner Medical Foundation, 1514 Jefferson Highway, New Orleans, LA 70121
*and *Veterans Administration Medical Center and Tulane University School of Medicine*
New Orleans, LA 70146

Received 17 September 1984

EHRENSING, R. H., A. J. KASTIN AND G. F. MICHELL. *Antagonism of morphine analgesia by prolyl-leucyl-glycinamide (MIF-1) in humans.* PHARMACOL BIOCHEM BEHAV 21(6) 975–978, 1984.—Prolyl-leucyl-glycinamide (MIF-1) has been observed to inhibit the analgesic effect of morphine in a series of animal studies. In the present study, the naloxone-like properties of MIF-1 were assessed in human subjects. Eight men received a capsule containing 60 mg of MIF-1 or placebo followed one hour later by a 10 mg intramuscular injection of moprhine in a double-blind, crossover design at two visits 4 weeks apart. Experimental pain was induced by the cold pressor test administered 45, 75, 120 and 180 min after the morphine. Each subject recorded severity of pain on a 100 mm line scale every 5 sec during the 120 sec his foot was immersed in the cold water tank and during the 60 seconds immediately following its removal. On a third visit, baseline values were measured in the absence of morphine, MIF-1 or placebo. Analysis of variance revealed that MIF-1 resulted in significantly higher scores (less analgesia) compared with placebo when measured at 45 and 75 min after morphine during the immersion phase and during all four times the subjects were evalulated during the removal phase. The results indicate that MIF-1 can act in humans as an opiate antagonist.

MIF-1 Morphine Analgesia Cold pressor pain Opiate antagonist Humans

PRETREATMENT with the tripeptide prolyl-leucyl-glycinamide (MIF-1) has significantly reduced the analgesic effect of morphine in a series of animal studies [5, 7, 8]. There have been no reported clinical trials of MIF-1 as a narcotic antagonist in humans. Accordingly, in this small pilot study, we now report the initial administration of MIF-1 to humans to test its effectiveness as an antagonist of morphine-induced analgesia against cold induced pain.

METHOD

Subjects

Eight male subjects, ranging in age from 21 to 32 years, volunteered with informed written consent to take part in the study and were paid $100 upon completion of their participation. Before entering the study, a physical examination, electrocardiogram, complete blood count (CBC), urinalysis, and blood chemistries were carried out for each subject and confirmed them to be in good health. The subjects did not use any analgesic medications during the week before entry into the study.

Apparatus

The cold pressor test was used to experimentally induce pain. The test was administered using a technique similar to that described elsewhere [6]. Briefly, an insulated 50-liter tank was divided in half with a wire screen so that ice cubes and water were confined to one-half and water alone remained in the other half. The temperature in the half of the tank containing only water was maintained to within one-half a degree of 0°C.

The subject was instructed to submerge his right foot in the side of the tank containing only water for 120 seconds. A 100 mm horizontal visual analog scale, similar to that described by Scott and Huskisson [11] was used to record severity of pain. The left end of the scale was marked "no pain" (zero rating) while the right end was marked "extreme pain"

[1]Presented in part at the XVth International Congress of the International Society for Psychoneuroendocrinology, Vienna, Austria, July 19, 1984.

(100 mm rating). At 5-sec intervals an 1800 Hz tone was presented for 0.1 second; this was the subject's cue to mark a vertical line through the point on the scale that best represented the degree of pain experienced at that time. After removal of his foot from the tank, the subject continued to mark the scale for an additional 60 seconds.

Experimental Design and Procedure

Appropriate FDA and institutional review and approval were obtained for the investigation. The study employed a double-blind, crossover experimental design. Each subject was required to come in for three visits, with a four-week interval between visits.

On visit 1, the subject was randomly assigned to receive a capsule containing either MIF-1 60 mg or an identically appearing placebo capsule followed one hour later by a 10 mg intramuscular injection of morphine. A 60 mg dose of MIF-1 was chosen because it has been demonstrated to be the most effective dose in the treatment of clinically depressed patients [3, 4, 13]. On visit 2, those subjects who received MIF-1 on visit 1 received placebo, and vice versa. The order of administration of MIF-1 and placebo was counterbalanced so that one-half the subjects received MIF-1 during visit 1 and the remaining half placebo. On visit 3, the subjects were tested without any MIF-1, placebo, or morphine, and these values used as the baseline scores. On visits 1 and 2, the cold pressor test was administered 45, 75, 120, and 180 min after the subject was injected with morphine. On visit 3, the subject was tested one time 15 minutes after arriving in the testing room. Observations during all visits were made between 8 a.m. and 12 noon.

Statistical Analyses

Treatment-by-treatment-by-subjects analyses of variances (ANOVA) were used to simultaneously compare the subjects' scores under the three different treatment conditions: MIF-1, placebo, and baseline. A separate ANOVA was performed for each of the four time intervals at which the subjects were tested during the immersion and removal phases of the cold pressor test. Winer F-tests for simple effects [14] were used to further assess any significant treatment × trials interactions.

RESULTS

The ANOVA performed on the data collected during the 120 sec immersion phase of the cold pressor test revealed that for each one of the four time intervals the subjects were tested there were significant main effects due to treatment: $F(2,14)=17.05$, $p<0.001$ at 45 min; $F(2,14)=10.06$, $p<0.01$ at 75 min; $F(2,14)=7.17$, $p<0.01$ at 120 min; and $F(2,14)=9.27$, $p<0.01$ at 180 min after injection of morphine. Significant treatment × trials interactions were revealed at 45 min, $F(46,322)=2.04$, $p<0.001$, 75 min, $F(46,322)=1.54$, $p<0.05$, and 120 min, $F(46,322)=1.85$, $p<0.01$, after morphine; this suggested that the MIF-1 and placebo scores might differ significantly from one another during the course of immersion for some, but not all, of the 24 trials tested. Accordingly, Winer F-tests for simple effects were used to further explore the interactions. These revealed that during the final one-third (40 sec) of immersion at 45 min and the final 25 sec at 75 min after morphine, treatment with MIF-1 led to significantly higher pain scores, i.e., less analgesia, compared with placebo (see Fig. 1 for significance levels).

Baseline pain scores were significantly higher than those of both MIF-1 and placebo at 45 min and at 75 min. Although the difference between the MIF-1 and placebo scores did not reach significance at 120 min after morphine, there was a difference in the relationship of each group to baseline values during the final 50 sec of immersion. During this time the scores for MIF-1 with morphine did not differ significantly from those of baseline without morphine, while the scores for the placebo with morphine were significantly lower than baseline. MIF-1 and placebo scores were not significantly different from each other at 180 min after morphine and both were significantly lower than baseline.

The results of the ANOVA performed on the data collected during the 60 sec removal phase of the cold pressor test were similar to those reported above for the immersion phase but contained several notable differences. Significant main effects of treatment were again revealed during all four times of observation: $F(2,14)=6.40$, $p<0.05$ at 45 min; $F(2,14)=5.19$, $p<0.05$ at 75 min; $F(2,14)=4.65$, $p<0.05$ at 120 min; and $F(2,14)=4.03$, $p<0.05$ at 180 min after injection of morphine. In addition, significant treatment × trials interactions were found at all four testing times: $F(22,154)=4.52$, $p<0.001$ at 45 min; $F(22,154)=3.70$, $p<0.001$ at 75 min; $F(22,154)=3.27$, $p<0.001$ at 120 min; and $F(22,154)=3.76$, $p<0.001$ at 180 min after morphine. Winer tests revealed highly significant differences between MIF-1 and placebo during the first 30 sec of the 60 sec removal period at 45 min and during the first 35 sec at 75 min after morphine (see Fig. 1 for significance levels). With the exception of the first (5 sec) trial at 120 min, the MIF-1 pain scores were significantly greater compared with placebo during the first 35 sec of removal and, at 180 min after morphine, the two groups differed significantly at 10 and 20 sec after removal.

In a comparison of baseline scores to MIF-1 and placebo at 45 min, with the exception of the first three trials (5, 10 and 15 sec) the MIF-1 group (with morphine) was not significantly different from baseline (without morphine) whereas the placebo (with morphine) scores were significantly lower compared with baseline from 5 through 45 sec after removal. MIF-1 scores did not differ significantly from those of baseline at 75 min after morphine except during the third trial (15 sec after removal). In contrast, the placebo scores were significantly lower than baseline during the entire initial 30 sec after removal. For both 120 and 180 min after morphine, the MIF-1 scores were significantly less than baseline 5, 10 and 15 sec after removal but could not be distinguished significantly from baseline during the remaining 45 sec. In contrast, the placebo group differed significantly from baseline during the initial 30 sec and 25 sec after removal at 120 and 180 min after morphine, respectively, indicative of a greater degree of analgesia when the subjects were treated with placebo than MIF-1.

DISCUSSION

The present study confirms our previously published findings [5, 7, 8] of the opiate-antagonistic effects of MIF-1 and extends them to a different type of pain and subject. The results reported here with human subjects are consistent with the concept that MIF-1 has naloxone-like properties and can, therefore, act as an opiate antagonist.

We observed significant decreases in analgesia (increases in perceived pain) in the eight subjects after treatment with MIF-1 and morphine as compared with treatment with placebo and morphine. It does not necessarily follow, however, that a different experimental design or the use of a

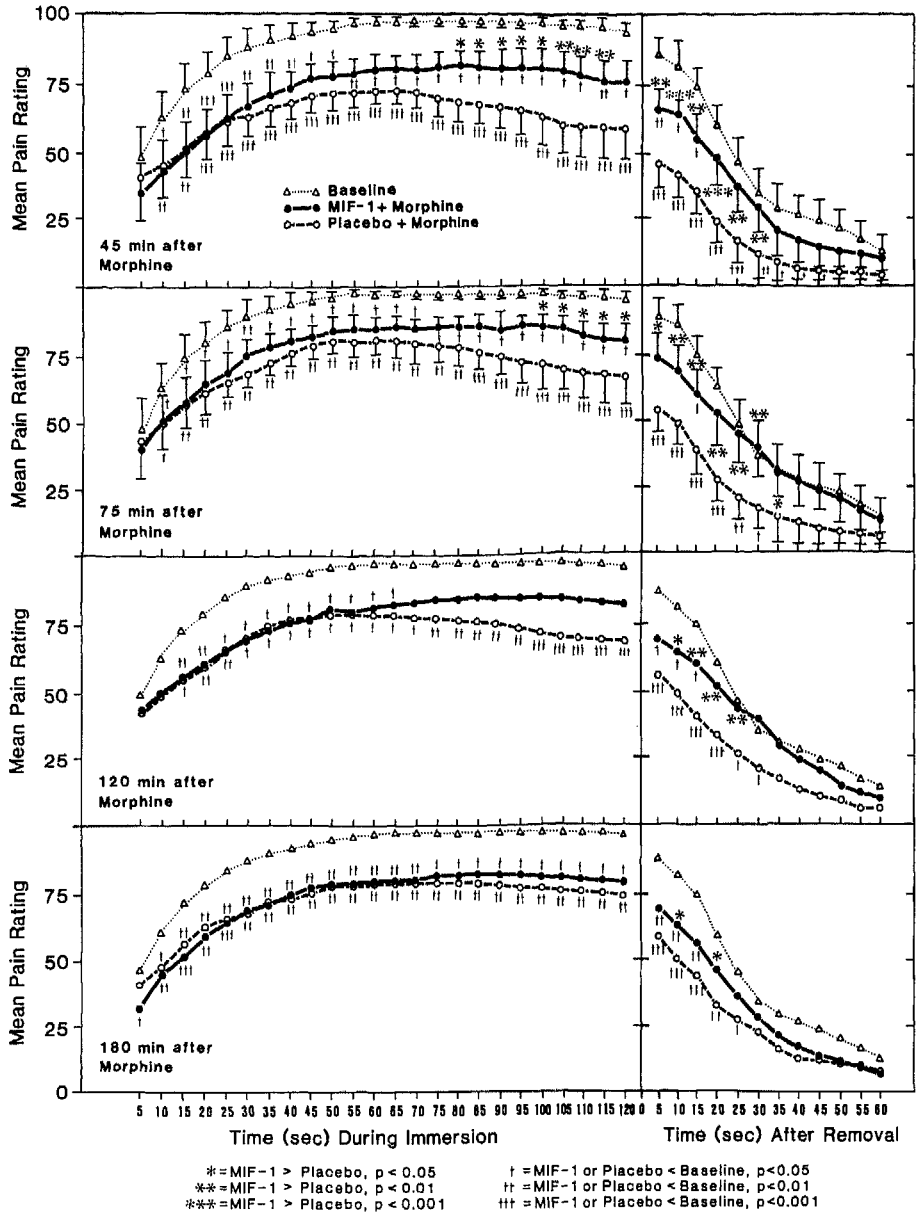

FIG. 1. Mean 100 mm line pain ratings as a function of immersion time and time after removal as measured during the four time intervals the subjects were tested (0 rating="no pain"; 100 mm rating="extreme pain").

different modality of pain should produce the same results. Our preliminary studies suggested the possibility that the experience of pain by the subject before the ingestion of MIF-1 might interfere with the opiate-antagonistic action of this tripeptide. Therefore, in this study we were careful not to expose the subjects to any pain before the administration of MIF-1 and morphine. This involved postponement of baseline readings to the third visit. Infliction of pain before a subject received MIF-1 might precipitate stress-induced endocrine and endogenous opiate changes that could compete with the effects of exogenously administered MIF-1. This possibility was supported by results subsequently obtained in the mouse tail-flick test in which significantly greater antagonism of morphine analgesia by MIF-1 occurred when basal readings were omitted before the injection of MIF-1 and morphine [9]. It is also possible that MIF-1 may not antagonize the analgesic effects of opiates to pain induced by electric shock, ischemia and means other than the thermal extremes of heat and cold. Schull, Kaplan and O'Brien [12] pointed to the different "quality, neural substrates, and/or adversiveness" of various types of painful stimuli in their discussion of the different effects they found for naloxone in the ischemic pain and cold pressor tests administered under different degrees of stress.

Some of the interrelationships of MIF-1, opiate peptides, ACTH and cortisol supporting a role for MIF-1 in the per-

ception of pain have been reviewed elsewhere [5]. Although it is not established if MIF-1 plays a physiological role as an endogenous opiate antagonist, such a naturally occurring naloxone-like substance might help explain variations in pain sensitivity and response to opiate analgesics. Davis *et al.* [1] have observed increased analgesia in depressed patients. Other investigators have suggested there is a similarity in the pharmacology of narcotic antagonists and anti-depressants [2]. MIF-1 has been reported to act as an anti-depressant [3, 4, 13] and now also is seen to have narcotic antagonist properties in humans.

As we have suggested previously [5], there are a number of possible mechanisms by which MIF-1 could reduce the analgesia associated with morphine. Although MIF-1 could act by preventing the action of morphine at opiate receptors, this does not appear to occur for mu and delta receptors [7, 8, 10]. MIF-1 could also block the release of endogenous opiate-like peptides (e.g., β-endorphin) or could indirectly influence opiate activity by acting through dopamine receptors or its own peptide receptor. In conclusion, this study is, to our knowledge, the first report of the antagonism of morphine analgesia in humans by a peptide.

REFERENCES

1. Davis, G. C., M. S. Buchsbaum and W. E. Bunney. Analgesia to painful stimuli in affective illness. *Am J Psychiatry* **136:** 1148–1151, 1979.
2. Doggett, N. W., H. Reno and P. S. J. Spencer. Narcotic agonists and antagonists as models for potential antidepressant drugs. *Neuropharmacology* **14:** 507–515, 1975.
3. Ehrensing, R. H. and A. J. Kastin. Melanocyte-stimulating hormone release inhibitive hormone as an anti-depressant: A pilot study. *Arch Gen Psychiatry* **30:** 63–65, 1974.
4. Ehrensing, R. H. and A. J. Kastin. Dose-related biphasic effect of prolyl-leucyl-glycinamide (MIF-1) in depression. *Am J Psychiatry* **135:** 562–566, 1978.
5. Ehrensing, R. H., G. F. Michell and A. J. Kastin. Similar antagonism of morphine analgesia by MIF-1 and naloxone in *Carassius auratus*. *Pharamcol Biochem Behav* **17:** 757–761, 1982.
6. Hilgard, E. R., H. MacDonald, G. Marshall and A. H. Morgan. Anticipation of pain and of pain control under hypnosis: Heart rate and blood pressure responses in the cold pressor test. *J Abnorm Psychol* **38:** 561–568, 1974.
7. Kastin, A. J., R. D. Olson, R. H. Ehrensing, M. C. Berzas, A. V. Schally and D. H. Coy. MIF-1's differential actions as an opiate antagonist. *Pharmacol Biochem Behav* **11:** 721–723, 1979.
8. Kastin, A. J., C. Nissen, J. E. Zadina, A. V. Schally and R. H. Ehrensing. Naloxone-like actions of MIF-1 do not require the presence of the pituitary. *Pharmacol Biochem Behav* **13:** 907–912, 1980.
9. Kastin, A. J., E. Stephens, R. H. Ehrensing and A. J. Fischman. Tyr-MIF-1 acts as an opiate antagonist in the tail-flick test. *Pharmacol Biochem Behav* **21:** 937–941, 1984.
10. Luciano, M. G., J. E. Zadina, A. J. Kastin and D. H. Coy. Mu and delta opiate receptors in rat brain are affected by GTP but not by MIF-1. *Brain Res Bull* **7:** 677–682, 1981.
11. Scott, J. and E. C. Huskisson. Graphic representation of pain. *Pain* **2:** 175–184, 1976.
12. Schull, J., H. Kaplan and C. P. O'Brien. Naloxone can alter experimental pain and mood in humans. *Physiol Psychol* **9:** 245–250, 1981.
13. van der Velde, C. D. Rapid clinical effectiveness of MIF in the treatment of major depressive illness. *Peptides* **4:** 297–300, 1983.
14. Winer, B. J. *Statistical Principles in Experimental Design*. New York: McGraw-Hill Book Company, 1971, pp. 529–532.