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Similar Antagonism of Morphine Analgesia by MIF-1 and Naloxone in *Carassius auratus*

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EHRENSING, R. H., G. F. MICHELL AND A. J. KASTIN. *Similar antagonism of morphine analgesia by MIF-1 and naloxone in* Carassius auratus. PHARMAC. BIOCHEM. BEHAV. 17(4) 757–761, 1982.—Prolyl-leucyl-glycinamide (MIF-1), the C-terminal tripeptide of oxytocin, and naloxone were administered intracranially (IC) to goldfish *(Carassius auratus)* in doses of 0.001, 0.01, 0.1, 1.0 and 10.0 mg/kg and compared to a diluent control group for their ability to reduce the effects of morphine (30 mg/kg IC) in an assay measuring analgesia to electric shock. Threshold levels of pain were determined by the voltage necessary to produce an agitated swimming response (ASR). Both MIF-1 and naloxone were found to significantly reduce the analgesic effects of morphine when compared to the diluent control group. Similar dose-response curves in an apparent sine-wave pattern were noted with both MIF-1 and naloxone when comparisons were made both at 20 minutes after administration of morphine and over the entire 150 minutes of the experiment. The results support the evidence that MIF-1 can act as an opiate antagonist.

MIF-1 Naloxone Morphine Analgesia Dose-response Goldfish

PROLYL-leucyl-glycinamide or MIF-1 is the C-terminal tripeptide of oxytocin and can be hydrolyzed from oxytocin by membrane bound exopeptidases in the hypothalamus [37]. In recent years, evidence has been presented from several laboratories that MIF-1 affects the actions of morphine in what may be interpreted as opposing ways. Results from several groups suggest that MIF-1 facilitates morphine [4, 31, 32] and β-endorphin [33] tolerance and dependence while those from another [2,38] suggest that it blocks morphine dependence.

We reported that 10, 1.0, 0.1 and 0.01 mg/kg intraperitoneal (IP) injections of MIF-1 and naloxone in mice were similar in reversing morphine-induced analgesia in the mouse tail flick assay [17,19]. Dunn and Ciofalo (personal communication) have found that MIF-1 has no analgesic properties when given alone in the mouse hot plate test and rat yeast paw pressure test, but is able to significantly reduce the analgesic effects of morphine when given as an oral pretreatment. In the present study, the effects of MIF-1 and naloxone were directly compared over a wide range of doses in an assay for measuring analgesia in the common goldfish *(Carassius auratus)*.

METHOD

Animals

Experimentally naive goldfish *(Carassius auratus)* were obtained from Ozark Fisheries, Stoutland, MO. The mean weight of the fish was about 30 g.

Apparatus and Procedure

Analgesia was measured according to the method of Jansen and Green [15]. Each fish was placed in a small plastic tank (28×17×12 cm) containing aerated water from a large community tank that housed the fish before they were tested. A power supply, specially constructed to produce a voltage that was continuously variable from 1 to a maximum of 22 volts, was connected to an electrical prod which was placed just caudal to the fish's dorsal fin. The stimulus at which it was assumed that pain was perceived by the fish was determined by gradually increasing the voltage until an agitated swimming response (ASR) appeared. The ASR was characterized by an abrupt twitch over the entire length of the fish's body. Baseline measures of this voltage, hereafter

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referred to as pain threshold levels, were taken immediately before the fish was pretreated with an injection of MIF-1, naloxone or diluent. This pretreatment occurred 30 minutes before each fish was injected with morphine (−30 min). Measures were also taken 15 minutes later (−15 min before morphine) and immediately before morphine (zero minutes), and then at 5, 10, 20, 30, 40, 60, 75, 90 and 120 minutes after morphine.

Fish were pretreated with 10 μl of synthetic MIF-1, naloxone or diluent injected into the cranial space over the optic tectum (referred to hereafter as intracranial or IC injections). The diluent consisted of saline acidified with 0.01 M acetic acid. Five doses (0.001, 0.01, 0.1, 1 and 10 mg/kg) of MIF-1 and naloxone, in addition to the diluent control group were used. Thus, there were 11 groups consisting of 5 fish in each group. All pretreatment substances were coded so that the investigator did not know their identity when determining the voltage at which the ASR appeared. Thirty minutes after the pretreatment, as outlined above, all fish received a 20 μl IC injection of morphine sulfate, 30 mg/kg. The Dunnett's Test and mixed analysis of variance were used to statistically compare the experimental groups to the diluent control group. The area under each curve from −30 to +120 minutes for all 55 fish was calculated using a Lasico Planimeter (Model N-10) to reflect the total development of analgesia during testing. The areas under the curves for the 5 fish in the diluent control group were compared with the corresponding areas for the 5 fish in each of the 10 experimental groups using an analysis of variance followed by the Dunnett's Test.

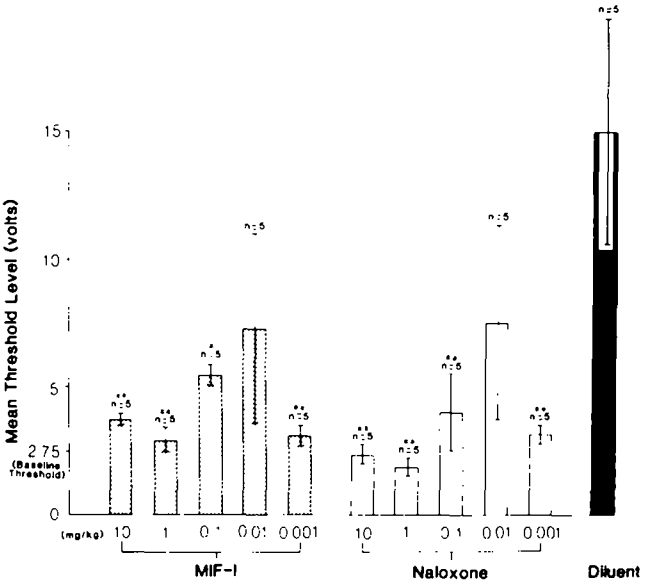

FIG. 1. Mean (±SEM) threshold voltage required to induce ASR in goldfish 20 min after morphine (30 mg/kg IC) and 50 min after MIF-1, naloxone or diluent IC (Dunnett's Test, MIF-1 and naloxone vs diluent, $*p<0.05$ and $**p<0.01$). The baseline threshold of 2.75 volts represents the combined mean threshold level for all 11 groups taken immediately before receiving the pretreatment injection.

RESULTS

Comparison of the results of all 5 doses of MIF-1 to the diluent control across total time tested (150 minutes) using a mixed analysis of variance revealed that MIF-1 significantly reduced the voltage required to induce the ASR after morphine, $F(5,24)=2.83$, $p<0.05$. An identical analysis performed on the 5 naloxone dose groups yielded no significant effect. However, a mixed analysis of variance performed only over the first 60 minutes of testing (30 min after injection of morphine) yielded a significant effect of naloxone, $F(5,24)=2.71$, $p<0.05$, compared to diluent. A fourth mixed analysis of variance comparing the 5 MIF-1 and 5 naloxone dose groups across total time tested revealed no significant differences.

The greatest blocking of analgesia with both MIF-1 and naloxone relative to diluent was observed at 20 minutes after morphine (Fig. 1). The 10, 1, 0.1, and 0.001 mg/kg dose groups of both MIF-1 and naloxone showed a significant reduction in analgesia at 20 minutes (Dunnett's Test, $p<0.01$, except MIF-1 0.1 mg/kg, where $p<0.05$). Neither of the two groups that received MIF-1 and naloxone at a dose of 0.01 mg/kg were significantly different from the diluent control. The significance of the effect of the 0.001 mg/kg dose of naloxone was unexpected in light of a lack of significant effect by the 0.01 mg/kg dose. These two doses of naloxone were coded and repeated with the investigator blind to the identity of the doses. The results were very similar to the original data: 0.01 mg/kg—n=5, mean threshold volts at 20 minutes=12.86±3.91, $p>0.2$; 0.001 mg/kg—n=5, mean threshold volts at 20 minutes=6.2±3.97, $p<0.025$.

The response profiles for the 5 MIF-1 and 5 naloxone groups were nearly identical at this time of 20 minutes after morphine. The Spearman rank order correlation coefficient yielded a significant correlation in mean threshold levels between MIF-1 and naloxone when paired by dose (RHO=+.90, $p<0.05$). This relationship can also be expressed in terms of absolute probabilities in that by chance alone the probability of each drug group having the five doses in a particular relative order of effect is 1/120 or 0.008. The probability of both drug groups having the same profile or relative order of effect is (1/120)(1/120)=0.000064. The results therefore support a very similar effect of approximately equimolar doses of MIF-1 (molecular weight=284.36) and naloxone (molecular weight=327.37) 20 minutes after administration of morphine.

Figures 2–6 show the development of analgesia in the diluent pretreated control group as compared to equal doses of MIF-1 and naloxone, each figure representing a different dose. The greatest and most persistent reduction in analgesia of all the groups over the 120 minutes tested after morphine occurred in fish injected with 1 mg/kg MIF-1 (Fig. 3). A significant reduction in analgesia was found 20, 30, 40, 60, 75 and 90 minutes after injection of morphine.

The results of the statistical comparisons made between the areas under the curves for analgesia for the 5 fish injected with diluent with the 5 fish in each of the experimental groups are shown in Fig. 7. This analysis also yielded what appeared to be sine-wave dose-response curves for both MIF-1 and naloxone. Both groups also had the identical relative order of effect of dose levels which, except for the switch in order of magnitude of the very similar effects of the 0.1 and 0.01 mg/kg doses, were the same as seen at 20 minutes (Fig. 1). Except for the 0.1 mg/kg doses of both MIF-1 and naloxone, all other groups of MIF-1 and naloxone that showed a significant reduction in analgesia at 20 minutes compared to the diluent also showed a significant reduction over the 150 minutes. An analysis of the area under the

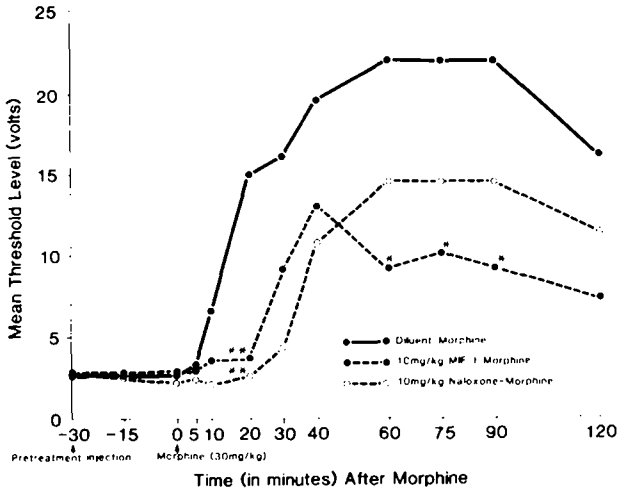

FIG. 2. Time course of development of analgesia assessed by ASR in morphine treated goldfish pretreated with diluent, MIF-1 (10 mg/kg) and naloxone (10 mg/kg) (Dunnett's Test, MIF-1 and naloxone vs diluent, $*p<0.05$ and $**p<0.01$).

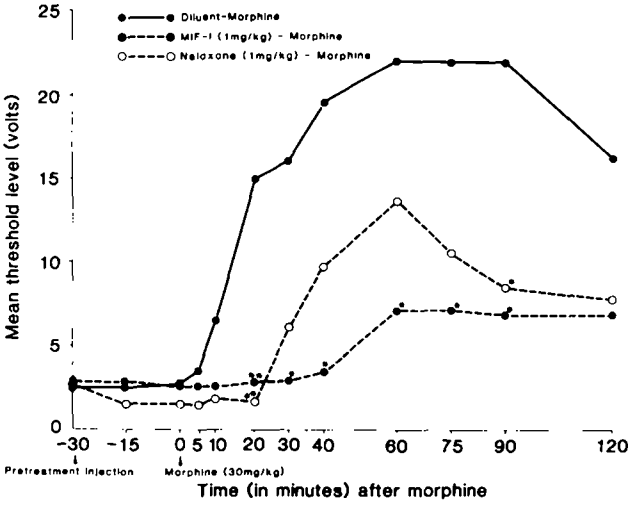

FIG. 3. Time course of development of analgesia assessed by ASR in morphine treated goldfish pretreated with diluent, MIF-1 (1 mg/kg) and naloxone (1 mg/kg) ($*p<0.05$ and $**p<0.01$).

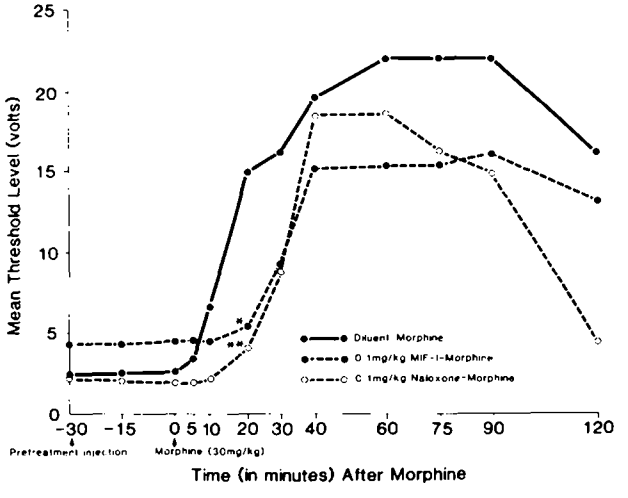

FIG. 4. Time course of development of analgesia assessed by ASR in morphine treated goldfish pretreated with diluent, MIF-1 (0.1 mg/kg) and naloxone (0.1 mg/kg) ($*p<0.05$ and $**p<0.01$).

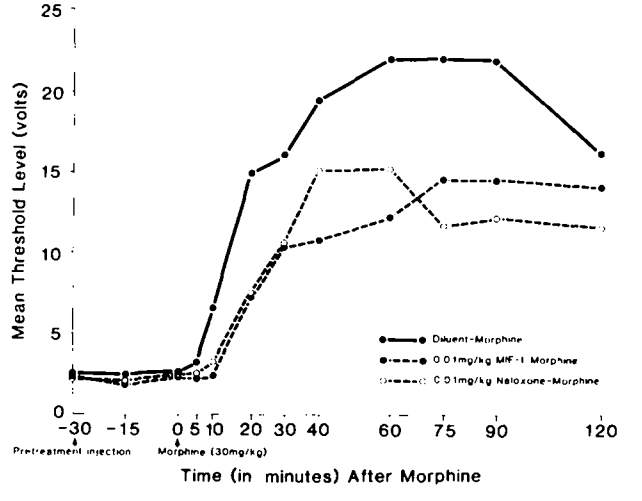

FIG. 5. Time course of development of analgesia assessed by ASR in morphine treated goldfish pretreated with diluent, MIF (0.01 mg/kg) and naloxone (0.01 mg/kg).

curves of the two naloxone dose groups which were repeated revealed again that the 0.001 mg/kg group was significantly different (mean area $=171\pm56$, $t=2.42$, $p<0.05$) from the diluent, while the 0.01 mg/kg group was not significantly different (mean area $=262\pm19$, $t=0.620$, $p>0.05$).

DISCUSSION

We have recently reviewed a series of animal and clinical studies that suggest curvilinear and biphasic dose-response curves may be characteristic of some peptide-mediated systems [20]. In particular, MIF-1 has been observed to have this curvilinear dose-response curve in the dopa-potentiation test in mice [26], in deserpidine antagonism in monkeys [27], in the reversal of swimming immobility in rats [16], in the reversal of morphine-induced catalepsy in rats [3], and in increased motor activity in monkeys [5]. In clinical studies, a similar dose-response curve after MIF-1 has been observed in mental depression [7, 9, 10], in the EEGs of healthy individuals [14] and perhaps in tardive dyskinesia [8].

In the present study, what appears to be a sine-wave dose-response curve was suggested by the return of significant reduction in morphine induced analgesia at a dose of 0.001 mg/kg of both MIF-1 and naloxone. This would support the hypothesis that MIF-1 and naloxone may block morphine analgesia through similar modes of action in this animal model. Olson *et al.* [24] have recently reported that MIF-1 acts like naloxone on fluid consumption in rats. With naloxone, they observed a significant effect in experimentally naive rats at 0.001 and 0.01 mg/kg doses, no effect

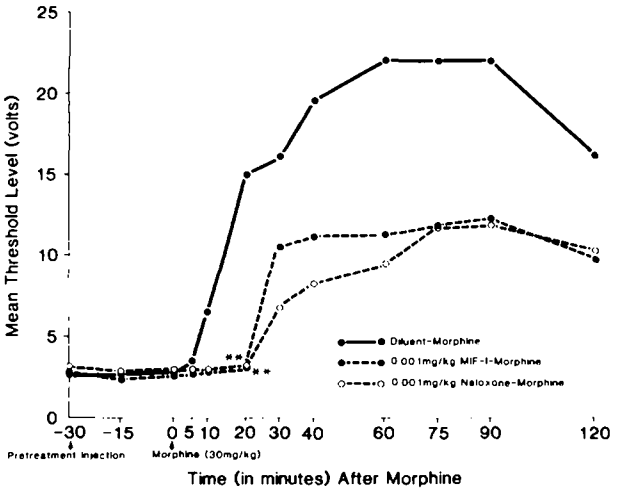

FIG. 6. Time course of development of analgesia assessed by ASR in morphine treated goldfish pretreated with diluent, MIF-1 (0.001 mg/kg) and naloxone (0.001 mg/kg) (**$p<0.01$).

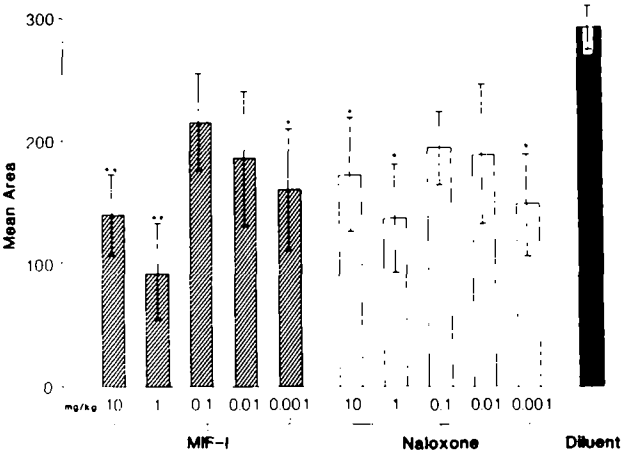

FIG. 7. Mean (±SEM) area under MIF-1, naloxone and diluent curves for analgesia from 30 to +120 min after morphine (*$p<0.05$ and **$p<0.01$).

at 0.1 mg/kg and then the opposite effect at higher doses. We also observed a significant effect at 0.001 mg/kg naloxone; the effect with the next highest dose, 0.01 mg/kg, was not significantly different from the diluent. In the mouse tail flick assay, however, the dose-response curve for MIF-1 and naloxone in blocking the effect of morphine was linear [19]. MIF-1 has also been observed to block the analgesic effects induced by an analog of enkephalin [17].

The present study did not investigate how long the effects of MIF-1 persist in reducing at least the initial analgesic effect of morphine given hours or days later. Although in this study we did not observe a significant degree of morphine antagonism with any of the doses at 120 minutes, at which time the analgesia was beginning to decline, this situation is not the same as if MIF-1 were administered, for example, 24 hours before morphine. If the action of MIF-1 persists for a long time, then its blockade of the effects of morphine could be misinterpreted as increasing tolerance to morphine and facilitating opiate dependence [31, 32, 33].

Interrelationships among MIF-1, opiates, ACTH, and cortisol have been demonstrated that could, by inference, be interpreted as supporting the hypothesis that MIF-1 may have a role in the perception of pain. MIF-1 has been reported after 20 minutes to significantly lower hypothalamic levels of β-endorphin/β-LPH when given intracerebroventricularly and to increase pituitary levels of β-End/β-LPH when given peripherally in rats [1]. ACTH and β-endorphin are derived from a common presursor [23], the so-named "pro-opiocortin" [30], have a parallel distribution in the brain [13], are both released in equimolar amounts by stress [28], and are both released by morphine [29]. Dexamethasone reduces the basal level of both peptides, and blocks the release of both to morphine and stress [21,29]. ACTH and the related peptide, α-MSH can, like β-endorphin, cause analgesia and behavioral effects when injected into the peri-aqueductal gray area [36]. MIF-1 has been reported to lower serum ACTH levels in Addison's Disease [34], but has not significantly affected ACTH levels in normal men [12] or those with Nelson's syndrome [6]. We have recently observed an apparent lowering of serum cortisols at 4 p.m. in depressed patients receiving MIF-1 in a dose of approximately 1 mg/kg at 9 a.m. [11]. Naloxone has been found to block the release of ACTH by morphine [29]; however, in normal men, naloxone has been observed to increase serum ACTH and cortisol [35]. MIF-1 does not have a naloxone-like action in the vas deferens assay and on food intake in VMH-lesioned rats [17], supporting the concept of dissociation of narcotic and behavioral actions of opiate peptides [18].

Among the ways MIF-1 could reduce the analgesia associated with morphine are by blocking the release of β-endorphin and/or other endogenous opiate-like peptides, by preventing the action of morphine on opiate receptors or by changing opiate receptors sensitivity through, for example, an effect on ACTH and cortisol levels. Plomp *et al.* [25] observed a significant decrease in morphine concentration in the brain 3 hours after morphine administered 1 hour after MIF-1, but no difference compared to saline 60 minutes after morphine. Luciano *et al.* [22] recently reported that MIF-1 does not bind to mu and delta receptors. It is more likely that, rather than acting directly on the opiate receptors, MIF-1 may act on its own peptide receptors and influence opiate receptors indirectly. Thus the results of the present study, through a direct comparison of effective doses of MIF-1 and naloxone, support the concept that MIF-1 may be an active opiate antagonist for analgesia.

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