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Tyr-MIF-1 Acts as an Opiate Antagonist in the Tail-Flick Test

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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(6) 937–941, 1984.—The naturally occurring brain peptide Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) was tested for its ability to block and reverse the actions of morphine in the tail-flick test. Injected peripherally either 10 minutes before or after morphine, Tyr-MIF-1, like MIF-1, was found to significantly reduce the antinociceptive actions of morphine on thermal pain. The results indicate that Tyr-MIF-1 may act, in part, as an endogenous opiate antagonist.

Analgesia Morphine Naloxone Brain Peptide

CONVINCING evidence now exists for the presence of Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) as an endogenous peptide. By radioimmunoassay (RIA), the presence of Tyr-MIF-1-like immunoreactivity was found in rat brain and pineal gland [10,11]. Gel filtration of pineal and hypothalamic tissue on Sephadex G-10 showed a peak eluting at the same position as the synthetic tetrapeptide, a position different from that of MIF-1 (Pro-Leu-Gly-NH_2) [10,11]. High performance liquid chromatography (HPLC) of brain tissue from which the pineal, hypothalamus, and pituitary had been removed confirmed recently the natural occurrence of Tyr-MIF-1 (unpublished observations). In addition, the presence of saturable, specific, high affinity binding sites for Tyr-MIF-1 has been demonstrated in rat brain; MIF-1 does not effectively compete for these sites [23]. In a test of passive avoidance, administration of Tyr-MIF-1 resulted in significant changes not seen with MIF-1 at the dose tested [8], whereas significant changes in reserpine-induced hypothermia in mice were seen with MIF-1 but not Tyr-MIF-1 [9]. Although brain enzymes can degrade Tyr-MIF-1 into MIF-1 [18], unpublished observations indicate that the effects of injected Tyr-MIF-1 are not mediated by conversion in blood to MIF-1. Together these results demonstrate that the occurrence of Tyr-MIF-1 cannot be accounted for by MIF-1 and raise the possibility that at least some of the actions of MIF-1 might not be shared by Tyr-MIF-1.

One of the actions MIF-1 can exert is that of an opiate antagonist. MIF-1 has been found to block the antinociceptive effects of opiates after acute [1, 6, 7, 12, 13] or chronic [3] administration. These actions did not seem to be mediated by mu or delta opiate receptors [13,16]. MIF-1 also exerted naloxone-like actions on body temperature [22] and drinking [19], but not on inhibition of the actions of Leu-enkephalin on blood pressure or heart rate [21]. It was not obvious, therefore, whether or not Tyr-MIF-1 would exert the same actions as MIF-1 in the tail-flick test.

METHOD

Preliminary Experiments

In preliminary experiments, the following variables were briefly examined: dose of MIF-1, dose of morphine sulfate, time after injection of MIF-1, time after injection of morphine sulfate, time of day, time exposed to heat, and intensity of heat. Diluent (0.9% NaCl acidified to 0.01 M with acetic acid) and, occasionally, naloxone (generously provided by Endo Labs) were used for comparison with MIF-1.

Experiment 1

Male, albino ICR mice weighing between 17–20 g at the time of the experiment were obtained from Charles River Laboratories (Wilmington, MA). Each mouse was tested in a 2.5×5 cm Plexiglas tube with 2 cm (1 cm from tip) of its exposed tail covering a small opening through which heat emanated from a nichrome thermal wire. A digital electronic timer was activated manually. Removal of the tail (tail-flick) from the grooved tray closed a photoelectric circuit and deactivated the timer. A rheostat maintained the heat so that the basal latency was about 4.5 sec. Each trial automatically ended at 17 sec.

Ten min before injection of morphine, each mouse received an intraperitoneal (IP) injection (10 ml/kg) of a freshly prepared, coded solution of MIF-1, Tyr-MIF-1, or diluent. Twenty mice were used at each IP dose of 0 (diluent), 0.001, 0.01, 0.1, 1.0, and 10.0 mg/kg; no mouse was used for more than one dose of any solution. Immediately before injection of peptide or diluent, a single basal determination of latency was made. Ten min later, morphine sulfate was injected at a dose of 12.5 mg/kg, IP and after 30 min (40 min after peptide or diluent), three consecutive readings of latency were taken. These latencies as well as the number of mice reducing their latencies to a mean of 12 sec or less (responders)

were compared by analysis of variance (ANOVA) followed by Duncan's New Multiple Range Test.

Experiment 2

This experiment was performed in the same way as Experiment 1 except that the morphine was injected 10 min before injection of the test solutions of peptide or diluent rather than 10 min afterwards. Readings were made 30 min after the second injection (40 min after the morphine). Only the single dose of 1.0 mg/kg of each peptide was used.

Experiment 3

This experiment combined the main features of each of the previous experiments in a balanced design. Tyr-MIF-1 and MIF-1 were injected at the dose of 1.0 mg/kg IP either 10 min before or 10 min after the morphine (12.5 mg/kg, IP) and the effects compared with the appropriate control group receiving diluent at the same times. Readings were obtained 30 min after injection of the morphine.

An additional study of a preliminary nature was tried because of the weak effect of the peptides in Experiment 2. It tended to show that different types of mice from different suppliers might be more responsive to MIF-1. Accordingly, in this experiment, Swiss-Webster mice were obtained from King Laboratories (Oregon, WI). In addition, the single basal reading for each mouse was omitted. It had been found in Experiments 1 and 2 that the basal values did not significantly (ANOVA) differ among groups and did not significantly (analysis of covariance) affect the subsequent response. Moreover, a recent pilot study in humans raised the possibility that the effects of a basal reading might interfere with the actions of MIF-1 in blocking morphine-induced analgesia [6].

Experiment 4

Since it appeared that the peptides might have been more active in Experiment 3 than in the previous experiments, the differences between these experiments were further evaluated. Swiss-Webster mice from King Labs had been used in Experiment 3 whereas ICR mice from Charles River Labs had been used in Experiments 1 and 2. These two strains of mice were compared in Experiment 4 together with a group of ICR mice from King Labs. If a difference were found between the first two groups, the last group would help decide whether the difference was due to conditions at the suppliers or the strain of mice.

The second difference in Experiment 3 from Experiments 1 and 2 was the omission of the basal reading. In Experiment 4, half the mice from each supplier had a single basal reading taken before injection of MIF-1 (1.0 mg/kg, IP) or diluent, and half of the mice in each of these six groups did not have any basal readings. In addition, half of each of the resulting 12 groups had MIF-1 or diluent injected 10 min before the morphine (12.5 mg/kg, IP), as in Experiment 1, and half of the groups received the test solutions 10 min after the morphine, as in Experiment 2. All readings were taken 30 min after the morphine, as in Experiment 3. The design, therefore, was similar to that of Experiment 3 with the addition of tests of the two variables of strain (and supplier) and basal readings. Since this design resulted in 24 groups with the use of only one peptide, MIF-1 was chosen because of our much larger supply of this tripeptide than the tetrapeptide. Five mice were tested in each of the 24 groups.

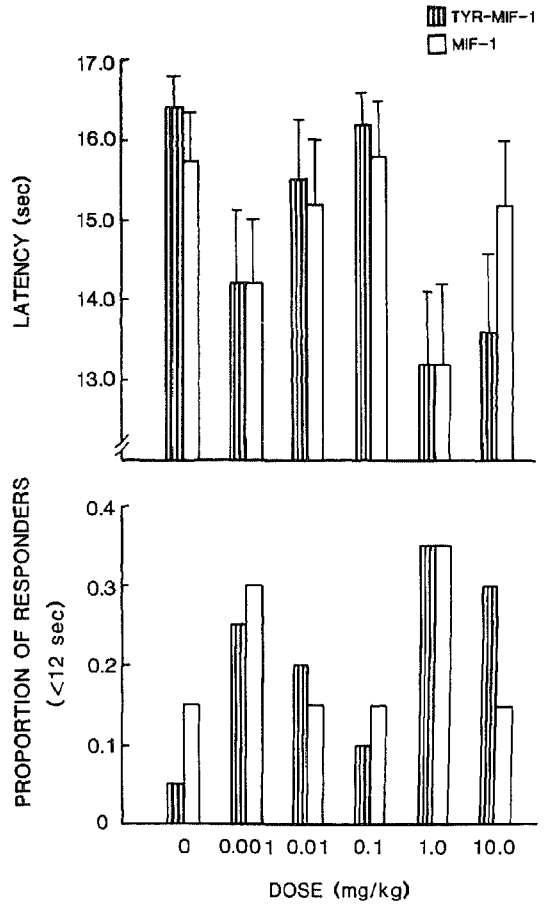

FIG. 1. Mean (±SEM) latency (top) and proportion of responders (<12 sec) (bottom) in tail-flick test (maximum: 17 sec; baseline: 4.5 sec) of mice after injected with Tyr-MIF-1 (striped bars) or MIF-1 (open bars) 10 min before morphine (12.5 mg/kg, IP). Dose 0 indicates diluent groups.

RESULTS

Preliminary Experiments

The preliminary studies involved injection of MIF-1 before the morphine. These indicated that great care must be used to obtain the optimal conditions for demonstration of the effects of MIF-1 in this system. The relationship between the amount of heat and dose of morphine appeared particularly critical. No differences in response during the morning or afternoon were obvious for peptide or naloxone, but naloxone was more potent than MIF-1 at each dose tested. Since the molecular weight of Tyr-MIF-1 (447; 508 as the acetate salt) is greater than that of MIF-1 (284) or naloxone (328), less of the tetrapeptide was injected at any dose (mg/kg).

For each mouse, there was very little variation among the three readings of latency 30 min after the morphine, a time suitable for demonstration of anti-opiate effects. Testing was restricted to only this one time, rather than at several additional times, in order to prevent thermal injury to the tail. This enabled a longer cut-off time to be used so that the available range for reduction of latencies would be maximized. Evidence that this procedure did not induce

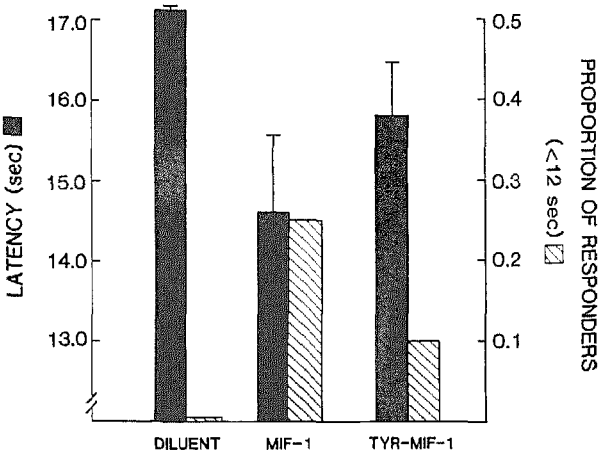

FIG. 2. Mean (±SEM) latency (solid bars) and proportion of responders (hatched bars) in mice injected with Tyr-MIF-1 or MIF-1 (1.0 mg/kg, IP), or diluent, 10 min after morphine (12.5 mg/kg, IP).

thermal injury was provided by results obtained in a separate group of mice injected with only morphine; 30 min later, immediately after the three tests at the 17 sec maximum were completed, they were injected with naloxone (1 mg/kg). This caused the latencies to return to the 4.5 sec baseline when tested 10 min later, a finding not possible if the tail had been burned.

The observation in these preliminary experiments that mice in each group seemed to flick their tails before 12 sec or not at all stimulated the decision to also compare the proportions of mice in these two categories and emphasize the occurrence of this all-or-none phenomenon. Responders were defined as those mice in which the analgesic effects of the morphine were reduced so that the latencies were <12 sec, whereas non-responders required >12 sec to flick their tails.

ANOVA is not as commonly used for analysis of binomial, all-or-none data, as it is for normally distributed samples. It is known, however, that "the sampling distribution of F is amazingly 'robust'; that is, it is insensitive to even flagrant violations of the (distribution) assumptions" [14] and is appropriate for use with binomial data under the conditions present in these experiments [16]. Therefore, we used ANOVA to also examine the proportion of responders (chi square analyses gave similar results).

Experiment 1

In this study involving injection of peptide before morphine, ANOVA for latencies showed no significant differences between MIF-1 and Tyr-MIF-1 and no interaction with dose, indicating that they were equally efficacious in this system. A significant main effect of dose was found, $F(5,228)=3.64$, $p<0.01$. Duncan's New Multiple Range Test showed significantly ($p<0.05$) shorter latencies for the groups receiving 1.0 mg/kg of MIF-1 and Tyr-MIF-1 as compared with the 40 mice receiving diluent, even though in a preliminary study 0.1 mg/kg appeared the most potent dose for MIF-1. When Tyr-MIF-1 was compared with its diluent group, significantly ($p<0.05$) shorter latencies were found for the mice receiving 1.0 mg/kg and 10.0 mg/kg as compared with either the diluent or the dose of 0.1 mg/kg. The means for latency are shown in the top section of Fig. 1. Statistical

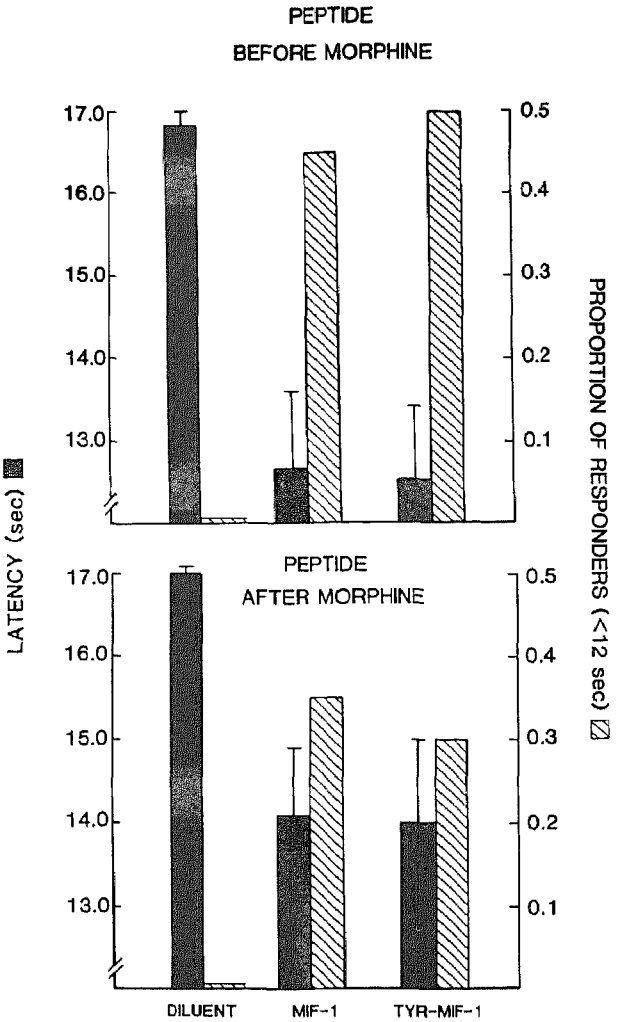

FIG. 3. Mean (±SEM) latency (solid bars) and proportion of responders (hatched bars) in mice injected with Tyr-MIF-1 or MIF-1 (1.0 mg/kg, IP), or diluent, 10 min before (top) or after (bottom) morphine (12.5 mg/kg, IP).

tests of the proportion of responders showed that only the dose of 1.0 mg/kg Tyr-MIF-1 resulted in significantly ($p<0.05$) fewer responses than diluent; these results are shown in the bottom section of Fig. 1.

Experiment 2

In this study involving injection of peptide after the morphine, ANOVA for the latency scores showed a significant main effect for treatment, $F(2,57)=3.50$, $p<0.05$. Duncan's New Multiple Range Test revealed that this effect was accounted for by the significantly ($p<0.05$) shorter latencies and increased proportion of responders for the mice receiving MIF-1 as compared with diluent (Fig. 2). No reliable differences were found between Tyr-MIF-1 and diluent or between the two peptides.

Experiment 3

ANOVA for latencies revealed a significant main effect of treatment, $F(2,114)=13.33$, $p<0.0001$. There was a tendency

($p=0.089$) toward a main effect of the order of treatment (peptide before or after morphine), but no significant interaction of treatment by order. Both Tyr-MIF-1 and MIF-1 caused a significant reduction in latencies (Fig. 3, top) for tail-flick as compared with their diluent controls when given 10 min before morphine ($p<0.001$ for both peptides) and when given 10 min after morphine ($p<0.05$ for both peptides). In neither experimental situation was there a significant difference between the effects of each peptide.

ANOVA for proportion of responders also revealed a significant effect of treatment, $F(2,114)=13.01$, $p<0.0001$. This was accounted for by the significantly higher porportion of responders among mice injected with either Tyr-MIF-1 ($p<0.001$) or MIF-1 ($p<0.01$) before the morphine and with either Tyr-MIF-1 ($p<0.05$) or MIF-1 ($p<0.01$) after the morphine, as compared with their respective control groups receiving diluent (Fig. 3, bottom). The effect of order of injection on the proportion of responders was not statistically significant.

Experiment 4

ANOVA for latencies in the 24 groups of 5 mice each revealed a significant main effect of treatment, $F(1,48)=13.48$, $p<0.001$, reflecting the lower latencies in mice treated with MIF-1. There was no significant main effect of strain or order in which morphine was administered. There was a tendency for a main effect of the presence or absence of basal reading, $F(1,48)=3.10$, $p=0.08$, and the interaction of treatment by basal reading was significant, $F(1,48)=4.35$, $p<0.05$, indicating that the measurement of basal latency changed the response to MIF-1, but not to diluent.

Although the main effect of strain was not significant, post-ANOVA tests showed that a substantial proportion of the variance resulting in the significant interaction of treatment by basal reading was contributed by the animals from King Labs, particularly the Swiss-Webster mice. In these groups, responsiveness to MIF-1 was clearly affected by the presence or absence of the basal measurement. The lowest mean latency (9.8 sec) was in the group of Swiss-Webster mice given a pre-morphine injection of MIF-1 without a basal reading. When measured both for latency and proportion of responders, this group was significantly ($p<0.001$) different from both the corresponding group injected with diluent and the group given MIF-1 with a basal reading. The difference between groups receiving and not receiving a basal reading was also highly significant ($p<0.01$) for the latency and proportion of responders in the Swiss-Webster mice given post-morphine MIF-1 and for the proportion of responders in the King Labs ICR group given post-morphine MIF-1. While there was a statistical tendency ($p<0.1$) for basal reading to affect responsiveness to MIF-1 in all but one of the remaining King Labs ICR groups, none of the measurements on Charles River ICR groups showed this tendency. In contrast to these effects of the basal measurement in the MIF-1 treated animals, none of the differences between groups receiving and not receiving a basal measurement were significant in diluent-treated animals, a result consistent with the significant interaction of treatment by basal reading.

DISCUSSION

The results show that Tyr-MIF-1 can interfere with the antinociceptive effects of morphine in the tail-flick test. In this respect, it acts like naloxone. Unlike naloxone, however, Tyr-MIF-1 has been detected in the brain where it could function, in part, as an endogenous opiate antagonist. Just as there are several endogenous opiate peptides, it is reasonable to expect that there are several endogenous opiate antagonists. These agonists and antagonists may exist in a delicate balance, perhaps partially explaining the necessity of careful adjustment of the experimental conditions to demonstrate the effect.

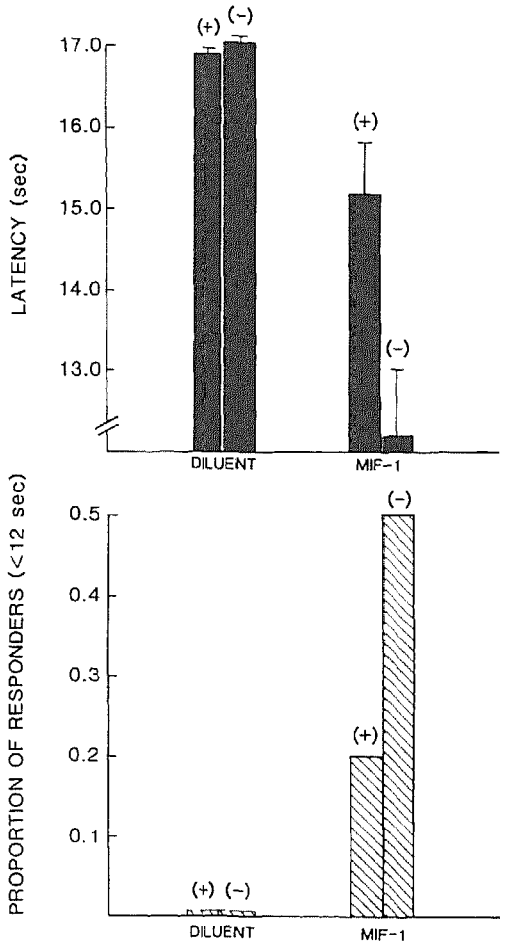

FIG. 4. Mean (±SEM) latency (top) and proportion of responders (bottom) for tail flick response after injection of MIF-1 (1 mg/kg, IP) or diluent in mice receiving morphine (12.5 mg/kg, IP). The "+" indicates groups in which a single basal reading was obtained before any injection; the "−" indicates groups in which the basal reading was omitted.

MIF-1 was again observed to reduce the actions of morphine in the tail-flick test, but it seemed less effective than on previous occasions in our laboratory [12,13]. Preliminary experiments ruled out differences in the dose of MIF-1, dose of morphine, cut-off time, and time after injection of morphine as the responsible factors; seasonal variables including temperature and humidity were not controlled. The omission of a single basal reading, suggested by a pilot study in humans [6], significantly improved the effect of peptide. This may indicate the importance of minimizing stress, and possibly the release of endogenous opiates and ACTH, for the detection of anti-opiate effects. The present studies were

performed in a laboratory temporarily located in a prefabricated structure more susceptible to noise and other environmental stimuli than in the permanent structure used in the previous tail-flick tests.

Although there was no significant effect of strain, there were some statistically reliable indications that mice from King Labs, particularly the Swiss-Webster strain, were more responsive to treatment with MIF-1, especially when the basal measurement was omitted. Limitations on the size and cost of the experiment, however, precluded a full factorial design with regard to strain and supplier. Furthermore, other factors, including shipping conditions, may be subsumed under the variable "supplier."

It is possible that in other experimental models, MIF-1 and Tyr-MIF-1 may act as stronger opiate antagonists. Since MIF-1 does not exert sufficient effects on mu and delta opiate receptors to explain its actions [13,16], however, it should not be expected to act exactly like naloxone in every situation.

MIF-1 has been found to exert unusual dose-response relationships in several laboratory [2, 6, 14, 20, 22] and clinical [4,5] studies. Since MIF-1 was the first peptide for which this phenomenon was described, the expression "inverted-U" was adopted to emphasize its occurrence. It is apparent, however, from Fig. 1 that more complex dose-response relationships may occur. These also have been noted in several of our unpublished studies (with M. V. Graf) with delta sleep-inducing peptide (DSIP). Whether these relationships, or the all-or-none type of response observed in the tail-flick test, involve the physicochemical forms in which peptides circulate and penetrate the blood-brain barrier is not clear.

The order in which the peptides and morphine were administered did not seem to reliably influence the results at the times tested. A differential effect might have occurred if longer times between injection of the compounds had been tested. Although Tyr-MIF-1 but not MIF-1 was effective at the dose of 10 mg/kg in Experiment 1 in which the peptide was administered before morphine, and MIF-1 but not Tyr-MIF-1 was effective in Experiment 2 in which peptide was administered after morphine, the effects of the two peptides were almost identical in Experiment 3 in which both peptides were tested in both situations in a balanced design, and there was no effect of order of injection in Experiment 4. Thus, the results demonstrate that both MIF-1 and Tyr-MIF-1 can act like opiate antagonists by interfering with the antinociceptive actions of morphine on thermal pain.

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