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Tyr-MIF-1 and MIF-1 are Active in the Water Wheel Test for Antidepressant Drugs

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KASTIN, A. J., D. A. ABEL, R. H. EHRENSING, D. H. COY AND M. V. GRAF. *Tyr-MIF-1 and MIF-1 are active in the water wheel test for antidepressant drugs.* PHARMACOL BIOCHEM BEHAV 21(5) 767–771, 1984.—MIF-1 [Pro-Leu-Gly-NH_2] and Tyr-MIF-1 [Tyr-Pro-Leu-Gly-NH_2] were tested in a system in which antidepressant drugs are known to result in increased wheel turning as mice attempt to escape from a small tank of water. One hr after injection, both peptides were found to cause a significant increase of the number of rotations of the wheel at doses as low as 0.01 mg/kg IP, the dose-response pattern for MIF-1 resembling an inverted-U. DSIP and morphine, by contrast, decreased the number of rotations. Under the conditions tested, neither MIF-1 nor Tyr-MIF-1 reversed the effect of morphine. The results demonstrate that MIF-1 and Tyr-MIF-1 are active in another test for antidepressants.

Depression Peptides Despair Lethargy Passiveness Immobility Swimming

AFTER MIF-1 [Pro-Leu-Gly-NH_2] was shown to be active in some of the same neuropharmacological tests [6, 7, 17, 18, 19, 25, 28] in which tricyclic antidepressants were also effective [1, 5, 16, 26], it was tried with some success [2, 3, 27] in the treatment of mental depression. As observed in the initial animal studies [17,18], an inverted U-shaped dose-response curve also seemed to occur with the depressed patients [2], indicating the caution required for clinical evaluation of the effective dosage of the peptide.

When Porsolt *et al.* [20] proposed a new animal model for antidepressants, we tried MIF-1 in this system [11]. Low doses (0.1 mg/kg IP), but not higher doses, of MIF-1 acted like amitriptyline in reversing the immobility and apparent lethargy of animals swimming in an inescapable narrow cylinder. Although the model had the advantage of not being influenced by the anxiolytics and tranquilizers tested [20], it seemed to be affected by several other compounds including antihistamines that were not antidepressants [24,29].

Nomura *et al.* recently proposed a modification of the method of Porsolt *et al.* [20] that responded to all antidepressants tested but not to tranquillizers, anticholinergics, or antihistamines [14]. Rather than having an observer distinguish between those movements of an animal necessary to keep its head above water and those movements designed for escape [20], the procedure of Nomura *et al.* [14] only recorded rotations of a small wheel turned by the animal while it attempted to escape during the period of "behavioral despair" 3–6 min after being placed in the water.

In addition to testing the tripeptide MIF-1 in this modified system, we also wanted to test the effects of the structurally related Tyr-MIF-1 [Tyr-Pro-Leu-Gly-NH_2]. Tyr-MIF-1-like activity has been detected in brain tissue by radioimmunoassay [9], by gel filtration on Sephadex of extracts from hypothalamus [9], and most recently by HPLC in extracts of brain tissue from which hypothalamus, pituitary, and pineal were excluded (Kastin and Fischman, unpublished observations). In addition, high affinity binding sites that are saturable and specific have been found for Tyr-MIF-1 in brain tissue [31].

Accordingly, we tested the effects of Tyr-MIF-1 as well as MIF-1 in this new behavioral model for antidepressant drugs [14]. Since MIF-1 [10] and Tyr-MIF-1 (unpublished observations) can also reverse the analgesic effects of morphine in the tail-flick model, the effects of these peptides on the actions of morphine in the water wheel model were also examined. As a measure of specificity, another peptide [delta sleep-inducing peptide (DSIP)] unrelated to MIF-1 or Tyr-MIF-1 was tested.

METHOD

Mice

The procedure described by Nomura *et al.* was used [14]. Mice weighing 25–30 g were obtained from Charles River Labs, Wilmington, MA and housed under a 12 hr light-dark cycle (lights on at 0600 hr).

Apparatus

As described by Nomura *et al.* [14], a water tank (20×8×18 cm deep) was constructed with Plexiglas material. It contained a water wheel with 6 paddles at the level of the water. The number of rotations of the water wheel was counted by a magnetic counter (Heathkit IM-Y100) attached to the shaft of the wheel. Water at 25°C was put into the tank to a height of 9 cm. The entire apparatus was placed in a water bath maintained at a constant temperature of 25°C.

Test Substances

Morphine sulfate was the generous gift of Penick Corp.

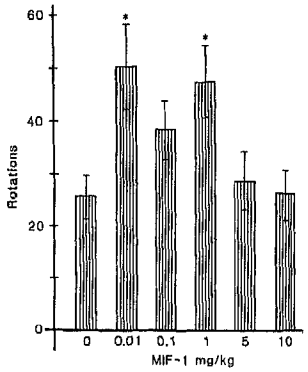

FIG. 1. Water wheel turning in mice during the standard time (3–6 min) after peripheral injection of MIF-1.

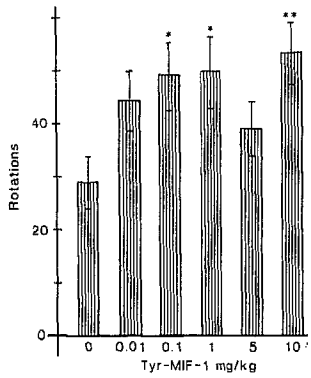

FIG. 2. Water wheel turning in mice during the standard time (3–6 min) after peripheral injection of Tyr-MIF-1.

(Lyndhurst, NJ). MIF-1 and Tyr-MIF-1 showed only one peak by high performance liquid chromatography (HPLC) and were >98% pure by amino acid analysis.

Test Procedure

One hr after injection of test material, each mouse was placed for 6 min into the water tank. Every group contained 10–20 mice. The number of rotations of the water wheel was counted separately for the first 3 min and the last 3 min. Only the values during the last 3 min were used as measures of escape behavior, as recommended by Nomura *et al.* [14].

Experiment 1—MIF-1. One hr before being placed in the water, mice were injected IP with one of six coded solutions containing the saline diluent (0 mg/kg body weight) or MIF-1 at the doses of 0.01, 0.1, 1.0, 5, or 10 mg/kg. Injection volume was 10 ml/kg.

Experiment 2—Tyr-MIF-1. This was identical to experiment 1 except that Tyr-MIF-1 was used instead of MIF-1.

Experiment 3—morphine. Morphine sulfate was injected IP at doses of 0 (diluent), 10, 20, 40, 50 or 60 mg/kg 1 hr before the mice were placed in the water.

Experiment 4—interaction of MIF-1/Tyr-MIF-1 and morphine. This experiment was performed the same way as Experiment 3 except that MIF-1 or Tyr-MIF-1 were injected IP concomitently with the morphine. Only one dose of peptide (1 mg/kg) and one dose of morphine (40 mg/kg) were used alone and in combination. The volume of each injection was 10 ml/kg.

Experiment 5—low doses of MIF-1 and Tyr-MIF-1. Each peptide was injected at doses of 0, 0.0001, 0.001, and 0.01 mg/kg in the same way as in Experiments 1 and 2.

Experiment 6—DSIP. Delta sleep-inducing peptide (DSIP) was injected one hr before the mice were placed into the water bath. The doses, determined on the basis of other experiments, were 0.05, 0.15, 0.3 and 1.0 mg/kg.

Statistics

Data in each experiment were compared by analysis of variance followed by Duncan's New Multiple Range Test. In separate analyses, Student's *t* test was used to compare the number of rotations of the wheel after injection of the diluent during the first 3 min of each experiment with the number of rotations during the second 3 min.

RESULTS

Consistent with the findings of Nomura *et al.* [14], in all experiments mice receiving diluent showed significantly fewer rotations during the second 3 min period than during the first 3 min ($p<0.01$, except Experiment 5 controls for Tyr-MIF-1, $p<0.05$). Although morphine produced its significant effects predominantly during the first 3 min of testing, none of the test peptides produced significant changes in activity during this period. A number of significant changes, however, were observed during the second 3 min period, and these are described below.

Experiment 1—MIF-1 (Fig. 1)

The higher doses of MIF-1 (5 and 10 mg/kg) failed to significantly increase the number of revolutions of the water wheel as compared to diluent during the second 3 min. Injection of 1 mg/kg and 0.01 mg/kg MIF-1 resulted in significantly ($p<0.05$) more counts than injection of 0, 5, or 10 mg/kg MIF-1. The dose of 0.1 mg/kg did not differ significantly from any other dose, including 0 mg/kg.

Experiment 2—Tyr-MIF-1 (Fig. 2)

Mice injected with Tyr-MIF-1 turned the wheel more than did mice injected with diluent alone. This reached statistical significance for the doses of 10 mg/kg ($p<0.01$), 1 mg/kg ($p<0.05$), and 0.1 mg/kg ($p<0.05$), but not for 5 mg/kg. The dose of 0.01 mg/kg Tyr-MIF-1 almost reached significance ($p=0.075$).

Experiment 3—Morphine Sulfate (Fig. 3)

During the second 3 min, the number of rotations of the water wheel were not changed to a statistically significant extent by injection of morphine one hr earlier.

During the first 3 min, however, all doses of morphine resulted in a significant ($p<0.01$) reduction in the number of revolutions of the water wheel. The larger doses of 40, 50 and 60 mg/kg also caused significantly ($p<0.01$) fewer revolutions than did the smaller doses of 10 and 20 mg/kg.

Experiment 4—Interaction of MIF-1, Tyr-MIF-1 and Morphine (Fig. 4)

In this experiment, Tyr-MIF-1 (1 mg/kg) and morphine

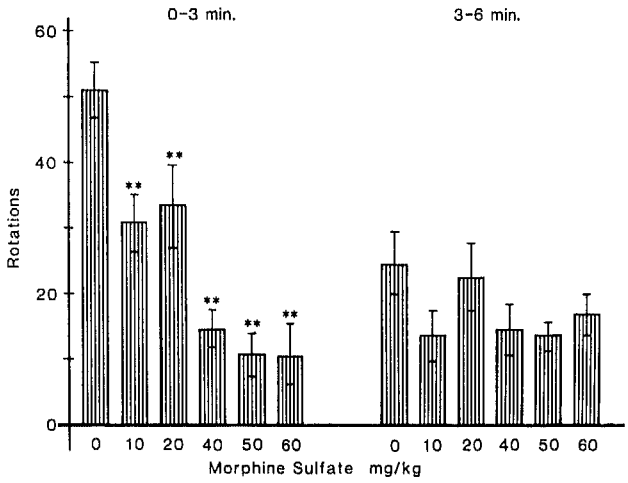

FIG. 3. Water wheel turning in mice during the initial and final 3 min periods after injection of morphine sulfate.

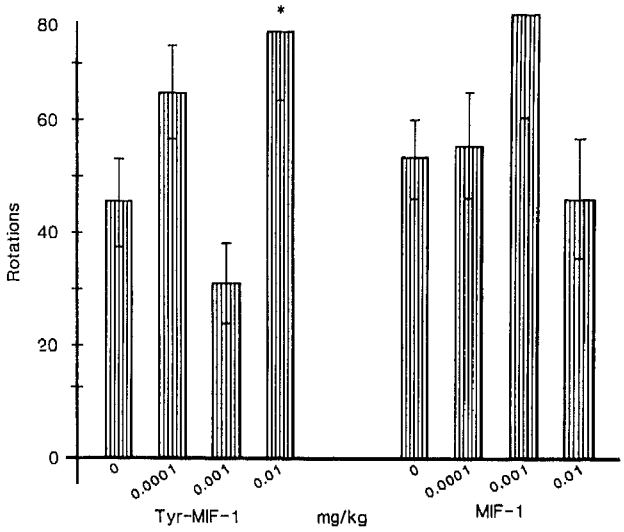

FIG. 5. Water wheel turning in mice during the standard time (3–6 min) after peripheral injection of low doses of MIF-1 and Tyr-MIF-1.

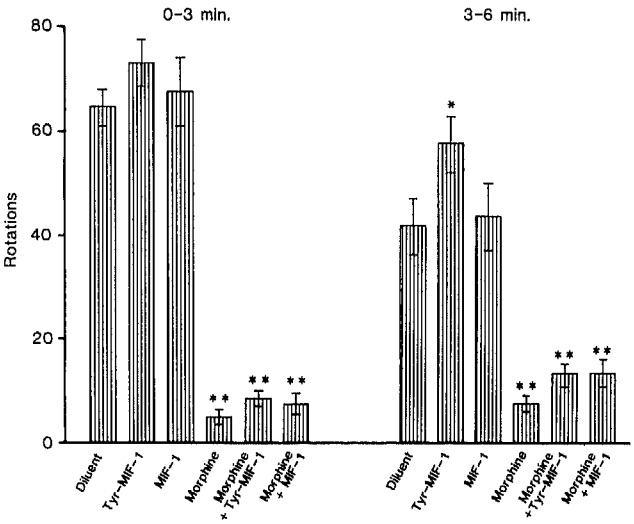

FIG. 4. Water wheel turning in mice during the initial and final 3 min periods after injection of morphine sulfate, MIF-1, Tyr-MIF-1, and their combinations.

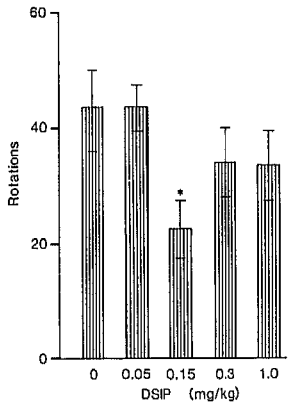

FIG. 6. Water wheel turning in mice during the standard time (3–6 min) after peripheral injection of DSIP.

(40 mg/kg) exerted effects by themselves that were reliably different from the effects of saline. Tyr-MIF-1 caused significantly more rotations ($p=0.14$ first 3 min, $p<0.02$ second 3 min) than saline whereas morphine caused significantly fewer rotations ($p<0.01$) during both periods. MIF-1, at the dose of 1 mg/kg, did not significantly change the number of rotations compared to diluent during either time.

When Tyr-MIF-1 was injected at the same time as morphine, the effect was not significantly different from the reliable effect exerted by morphine alone. Similarly, MIF-1 +morphine exerted the same effect as morphine itself. These results were seen during both periods of time. Preliminary results suggested that under the same experimental conditions naloxone (1 mg/kg) also failed to reverse these effects of morphine.

Experiment 5—Low Doses of MIF-1 and Tyr-MIF-1 (Fig. 5)

For Tyr-MIF-1 during the second period, the dose of 0.01 mg/kg resulted in significantly ($p<0.05$) more revolutions of the water wheel than did the 0 dose (diluent). The effect of the smallest dose, 0.0001 mg/kg did not reach statistical significance ($p=0.12$). The decreased number of revolutions associated with administration of the dose of 0.001 mg/kg was significantly ($p<0.05$) different from the other two doses of Tyr-MIF-1, but not from the 0 dose ($p=0.25$).

Experiment 6—DSIP (Fig. 6)

The overall effect of DSIP on rotations was statistically significant only during the second 3 min. All doses tended to

result in fewer turns than did diluent, but only the dose of 0.15 mg/kg reached statistical significance ($p<0.05$).

DISCUSSION

Administration of MIF-1 resulted in an increased number of rotations of the water wheel that represented a reversal of the immobility observed after injection of the diluent control (Fig. 1). This adds another test to the list of animal models in which MIF-1 and tricyclic antidepressants exert similar effects [1, 5, 6, 11, 16–18, 26, 28].

Tyr-MIF-1, shown by several techniques to exist in brain tissue [9,12], also increased wheel turning in mice (Fig. 2). Although Tyr-MIF-1 can be enzymatically degraded to MIF-1 [13], there is little evidence to support the idea that MIF-1 represents the only active form of Tyr-MIF-1. It has been shown previously [12] that MIF-1 does not effectively compete for the binding sites labeled with Tyr-MIF-1 in rat brain and Tyr-MIF-1 appeared to be more active in increasing wheel turning in mice than did MIF-1. This was most apparent in Experiment 4 involving the single dose of 1 mg/kg (Fig. 4) and in Experiment 5 involving doses of 0.01 mg/kg and lower (Fig. 5). In addition, Tyr-MIF-1 but not MIF-1 was active at the highest dose of 10 mg/kg (Figs. 1 and 2).

The lack of activity of the highest doses of MIF-1 in the water wheel test (Fig. 1) illustrates the inverted U-shaped dose-response curve first described for peptides in laboratory animals studied in tests considered by many investigators to represent animal models of depression as well as in clinical studies of mental depression [3, 6, 17, 18, 28]. It is not known whether this pattern would have become evident with Tyr-MIF-1 if higher doses had been tried. The experiments here indicate, however, that Tyr-MIF-1 has a broader range of effective doses than does MIF-1.

It also is not known whether some of the same factors resulting in the inverted U-shaped dose-response curve are involved in the relatively prolonged actions of MIF-1 and Tyr-MIF-1. These peptides were injected one hr before the mice were placed in the water, the same time interval used for the tricyclic antidepressants in the original description of the test [14]. Yet the half-time disappearance of MIF-1 in both rats [22] and humans [21] is only a few minutes. In the water wheel test, moreover, injections were made peripherally; this supports the initially controversial but now widely accepted concept that peripherally administered peptides can exert central effects [8].

In this new behavioral test for antidepressant drugs, Nomura *et al.* only used the results obtained from rotations of the wheel made during the second 3 min period [14]. The great activity during the initial 3 min period has been considered to represent attempts by the animal to escape from the water regardless of treatment. After this period, it appears that the untreated mouse "resigns itself" in a "state of despair" to the inescapable experimental situation [20]. Besides the slight activity involved in floating and clinging to the wheel, the main movements made by animals during the second 3 min period involved wheel turning [14]. This difference in activity between the two periods of time was supported by our finding that in each experiment the control groups injected with diluent made significantly fewer rotations of the water wheel during the second 3 min than during the first 3 min. This provided a suitable baseline for tests of the antidepressants.

During the first 3 min interval, MIF-1 and Tyr-MIF-1 did not exert significant effects. Similarly, nortriptyline, amitriptyline, clomipramine, zimelidine, nomifensine, and mianserine also did not cause significant changes during this initial period [14]. During the second 3 min, however, all of these antidepressants, as well as MIF-1 and Tyr-MIF-1, caused increased wheel turning. By contrast, neither DSIP nor morphine increased wheel turning during either period of time. On the contrary, DSIP significantly decreased the number of rotations during the second 3 min (Fig. 6), demonstrating that different peptides can exert opposite effects in this experimental system. This effect of DSIP was absent during the first 3 min, but it was during this initial period that the highly significant decrease in activity after each dose of morphine was seen (Fig. 4).

Decreased wheel turning after morphine also was seen in another experiment involving interactions with Tyr-MIF-1 and MIF-1. Although administration of Tyr-MIF-1 by itself resulted in significantly more rotations, the effect of the opiate was not altered by the peptide when they were administered together (Fig. 5). Since only one dose of each compound at one time of injection was tested, interpretation must be limited, but the results suggest that the effects of MIF-1 and Tyr-MIF-1 in the water wheel test do not depend on their actions as opiate antagonists. Naloxone-like effects of these peptides have been observed in several experiments [4, 10, 15, 30], although other situations have been described in which they do not act like naloxone [12, 23, 30].

Thus, the actions of MIF-1 and Tyr-MIF-1 in the water wheel test differ from those of morphine and DSIP. Effects are seen one hr after injection of these peptides in the periphery and strongly resemble the effects reported for antidepressants in the same system [14]. The results support the advisability of testing the members of the Tyr-MIF-1/MIF-1 family of peptides in mental depression.

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