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BRIEF COMMUNICATION

Some Analogs of Tyr-MIF-1 Affect Passive Avoidance Behavior but not Motor Activity in Rats

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HAYASHI, T., A. J. FISCHMAN, A. J. KASTIN AND D. H. COY. *Some analogs of Tyr-MIF-1 affect passive avoidance behavior but not motor activity in rats.* PHARMACOL BIOCHEM BEHAV 21(5) 809–812, 1984.—The effects of three analogs of the brain peptide Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) were tested in several behavioral tests after peripheral injection in rats. In the passive avoidance test, rats injected SC with 1 mg/kg Ala-MIF-1 or Phe-MIF-1 entered the second compartment of two-chamber shuttle box significantly faster than did rats receiving diluent. Leu-MIF-1 failed to produce significantly faster entry compared to diluent. None of the peptides significantly affected ambulation, rearing, or defecation. Flinch and escape thresholds were not affected by Ala-MIF-1, the only analog tested for this behavior. The results demonstrate that some analogs of Tyr-MIF-1 can exert behavioral effects similar to those exerted by the parent compound whereas other analogs resemble MIF-1 in being inactive under these experimental conditions.

Tyr-MIF-1 analogs Passive avoidance Flinch Escape Locomotion Behavior Peptides

THE apparent existence of Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) in rat brain as a substance distinguishable from MIF-1 (Pro-Leu-Gly-NH_2), a peptide with known CNS effects [8], has been demonstrated by radioimmunoassay (RIA) [6], receptor binding [11], gel permeation chromatography [6], and high performance liquid chromatography (HPLC) (unpublished observations). In both chromatographic systems the peptides have different elution positions, and in the RIA the antibody to Tyr-MIF-1 cross-reacts only 1.5% with MIF-1 [6]. MIF-1 does not effectively compete for the high affinity, saturable binding sites of Tyr-MIF-1 that have been demonstrated to exist in rat brain [11].

These findings and the structural similarity to MIF-1 suggest the possible involvement of Tyr-MIF-1 in behavioral and motor activities. Recently we have shown that Tyr-MIF-1 affects passive avoidance behavior but not motor activity in rats [4], but MIF-1 had no observed behavioral effect in these systems at the same dose. In order to further elucidate the molecular structural requirements for the behavioral effects of Tyr-MIF-1, a series of specifically designed analogs of Tyr-MIF-1 were prepared and the effects of peripheral injection of these compounds were investigated in several behavioral tests.

METHOD

Animals

Adult, male albino rats weighing about 200 g were obtained from Zivic-Miller Laboratories, Allison Park, PA. They were housed, 4 per cage, on a 12:12 light:dark cycle (lights on at 0700 hr) and laboratory chow and tap water were freely available.

Peptide Preparation

Ala-Pro-Leu-Gly-NH_2 (Ala-MIF-1), Leu-Pro-Leu-Gly-NH_2 (Leu-MIF-1), and Phe-Pro-Leu-Gly-NH_2 (Phe-MIF-1) were prepared by solid phase methods using benzhydrylamine resin to generate peptide amides after cleavage with liquid hydrogen fluoride and concomitant deblocking. The crude synthetic products were desalted by gel filtration chromatography on Sephadex G-10 (2.5×60 cm) eluted with 1.0 M acetic acid. For final purification, the desalted product was rechromatographed on the same column eluted with 0.02 M acetic acid. Thin layer chromatography of all three peptides revealed single spots in the following systems: n-butanol: acetic acid:water 4:1:5 (upper phase), ethyl ace-

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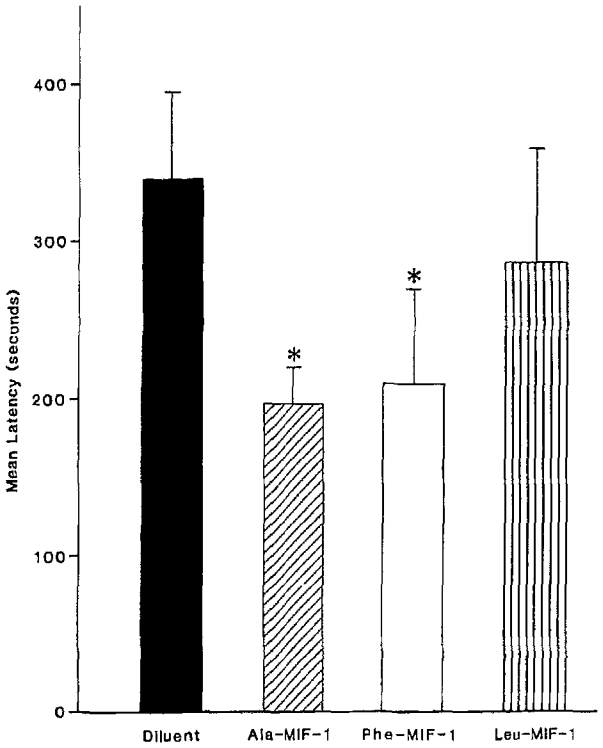

FIG. 1. Mean (±SEM) latencies for the passive avoidance trials on Day 5.

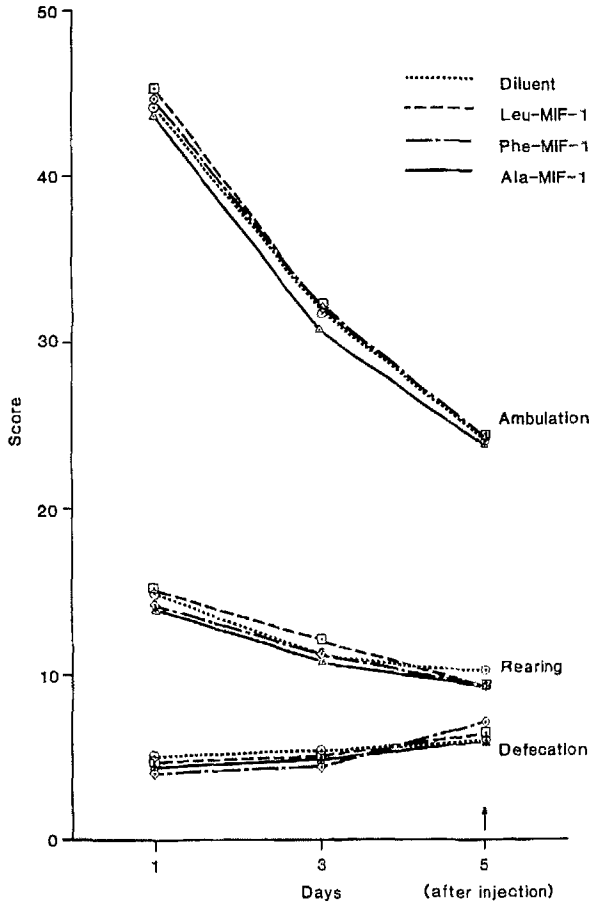

FIG. 2. Mean scores during the 3 min observation periods on Days 1, 3, and 5. Ambulation: number of grids crossed; rearing: number of hind-leg rearings; defecation: number of boli.

tate: acetic acid: n-butanol:water 1:1:1:1, and chloroform:methanol:water 8:5:1. Reversed phase HPLC (C-18 column, 0.45×15 cm) with a linear gradient from 2% methanol/0.1% TFA to 35% methanol/0.1% TFA showed a single peak for each peptide.

Procedures

On Days 1, 3, and 5 of a 5-day procedure, rats were placed in a two-chamber shuttle box (apparatus previously described [4]). On Days 2 and 4, no tests were performed. On Day 1, rats were observed in the box for 3 min, during which time ambulation (number of grids crossed), rearings, and defecations were recorded. This procedure was repeated on Day 3, but a single passive avoidance session was conducted at the end of the 3 min observation period. As each rat entered the lower compartment, a 0.2 mA electric shock was applied. The latency for Day 3 was recorded as the time elapsing between the point at which the animal was returned to the upper chamber and that at which he again had all 4 paws on the floor of the lower chamber. Rats having Day 3 latencies of less than 15 sec and the outliers from the tests of ambulation and rearing were excluded from further studies. If the latency to enter the lower compartment exceeded 15 min, the trial was terminated at that time.

On the fifth day, injections were made 15 min before the 3 min period of observation. Injections consisted of coded solutions of Ala-MIF-1 (1 mg/kg), Leu-MIF-1 (1 mg/kg), Phe-MIF-1 (1 mg/kg), or diluent (0.9% NaCl acidified to 1.0 M with acetic acid) in a volume of 1 ml/kg SC. The rats had been divided into 4 balanced groups based on the latencies determined on Day 3 and were randomly assigned to receive diluent or one of the peptides. After the observational tests were performed for 3 min, the rats were tested for passive avoidance as described above. The passive avoidance procedure was repeated in succession for a total of 3 sessions and the latencies recorded each time. The 5-day procedure was performed on 6 separate occasions, with 5–6 rats receiving each treatment (diluent or peptide) in every replicate.

The procedure for the Flinch/Escape test was performed on a separate set of experimentally naive rats as described above with the following changes. The "flinch threshold" was determined by recording the current (mA) at which the rats initially made withdrawal movements of their legs. As the current was further increased, the rats escaped the shock by entering the other chamber. The current at which this "escape" occurred was defined as the escape threshold. Flinch and escape thresholds were measured on Day 3 (no injection) and Day 5 (immediately after the 3 min observation period, 15 min after injection). Only Ala-MIF-1 and diluent were tested for this.

Statistical Analysis

The means were evaluated by analysis of variance followed by Duncan's new multiple range test.

RESULTS

A preliminary analysis of variance run on the Day 5 latencies from the passive avoidance experiments failed to reveal a significant effect of the days of replication and daily means were used in subsequent analyses.

Analysis of variance on these mean latencies yielded a significant main effect of peptide treatment, $F(3,18)=3.18$, $p<0.05$. As is shown in Fig. 1, the rats treated with Ala-MIF-1 and Phe-MIF-1 had significantly ($p<0.05$) shorter latencies than the diluent group. The latencies for rats treated with Leu-MIF-1 were not significantly different from those for the diluent group. A main effect of trials was also significant, $F(2,36)=85.33$, $p<0.001$, with latencies increasing over the 3 trials. No other effects were significant, including a separate analysis of variance made in order to test whether the rats had been appropriately separated into 4 groups based on their latency scores on Day 3.

Analysis of variance performed on the data for ambulation showed a significant main effect of trials, $F(2,352)=426.33$, $p<0.001$. This reflects the decreased motor activity over trials (Fig. 2). Analysis of variance on the data for defecation also showed a significant main effect of trials, $F(2,352)=26.91$, $p<0.001$, with number of boli increasing slightly over trials (Fig. 2). The data for rearing also showed a reliable effect of trials, $F(2,352)=56.64$, $p<0.001$, the number of rearings decreasing over trials (Fig. 2). No significant effects of peptide treatment were found for ambulation, defecation, or rearing.

Analysis of variance of the results obtained in the tests of flinch threshold and escape threshold either before (Day 3) or after (Day 5) injection failed to indicate any significant effects for Ala-MIF-1. The mean (±SEM) flinch thresholds (mA) for Ala-MIF-1 were 0.119±0.001 (Day 3) and 0.120±0.002 (Day 5) and for diluent were 0.119±0.001 (Day 3) and 0.119±0.001 (Day 5). The mean escape thresholds (mA) for Ala-MIF-1 were 0.160±0.002 (Day 3) and 0.164±0.004 (Day 5) and for diluent were 0.158±0.002 (Day 3) and 0.163±0.003 (Day 5).

DISCUSSION

Ala-MIF-1 and Phe-MIF-1, but not Leu-MIF-1, reliably influenced behavior in the passive avoidance paradigm and were clearly effective at a dose of 1 mg/kg. The decreased latencies after Ala-MIF-1 and Phe-MIF-1 suggested either interference with the original learning that occurred on Day 3 or attenuation of the effects of pain from the shock. The observation that neither flinch nor escape threshold was affected by Ala-MIF-1, the only analog tested for this, argues against a pain-attenuating effect. The decreased latencies over trials is consistent with most studies in which exploratory behavior and general activity are measured. Furthermore, the fact that ambulation, rearing, and defecation were not differentially modulated by peptide treatment indicates that the results with passive avoidance were not a function of the influence of the peptides on motor activity or emotionality.

Since the Tyr-MIF-1 analogs are structurally related to MIF-1 and it has been shown that Tyr-MIF-1 can be converted to MIF-1 by a specific brain aminopeptidase [9], it is likely that the analogs (particularly Phe-MIF-1) may also be substrates for this enzyme. This possible conversion would argue for similar actions of MIF-1, Tyr-MIF-1, and Tyr-MIF-1 analogs. Under the conditions of the present study, none of the peptides tested affected ambulation or rearing. MIF-1 has been shown not to affect motor activity [4, 7, 10]; the present results extend the observation to Ala-MIF-1, Phe-MIF-1, and Leu-MIF-1. However, the reliable effect of Ala-MIF-1, Phe-MIF-1, and Tyr-MIF-1 [4], but not MIF-1 [4] or Leu-MIF-1 on the passive avoidance response emphasizes the dissociation in actions that may occur between closely related peptides.

The results of this study provide new insight into the molecular structural requirements for the behavioral effects of Tyr-MIF-1 analogs. It appears that a tetrapeptide structure is required to affect passive avoidance behavior at the dose tested. The results with Phe-MIF-1 are not surprising, considering the close structural similarity of phenylalanine and tyrosine side chains. Similarly, the lack of effect of Leu-MIF-1 in the passive avoidance model is consistent with previously reported differences in the spatial orientation of leucine and tyrosine side chains in peptides [1–3, 5]. The observation that Ala-MIF-1 has substantial activity in the model suggests that an aromatic side chain is not essential for passive avoidance activity. The observation that Tyr-MIF-1 [4] but not Ala-MIF-1 affects flinch and escape thresholds, however, suggests more stringent structural constraints for this activity.

Changes in peptide dose might be expected to result in different patterns of response among the analogs. The inverted U-shaped dose-response pattern that has been observed in several systems with MIF-1 [8] raises the possibility that different doses of the analogs might cause similar effects.

Regardless, the results show that Ala-MIF-1 and Phe-MIF-1 can affect behavior and perhaps memory. This action is not readily explained by effects on motor activity, emotionality, or sensitivity to electric shock. The results also show that some of the analogs can act like Tyr-MIF-1 but, under these experimental conditions, unlike MIF-1.

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