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Tyr-MIF-1 attenuates antinociceptive responses induced by three models of stress-analgesia

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1 Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2), a biologically active brain peptide, has previously been shown to antagonize the analgesia induced by morphine.

2 In this report experiments are described in which mice were tested on the hot-plate in three models of antinociception – shock, novel environment, and warm-water swim – after the administration of various doses of Tyr-MIF-1 without any exogenous opiates.

3 The peptide reduced the antinociception produced by all three methods of inducing endogenous antinociception.

4 These results add further support for the existence of peptides like Tyr-MIF-1 that act as opiate antagonists.

Introduction

Increasing evidence suggests that the peptide Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) is a biologically active brain peptide with actions on CNS-mediated activity. Tyr-MIF-1 immunoreactivity in the brain is detectable by radioimmunoassay (RIA) and high performance liquid chromatography (h.p.l.c.) techniques (Kastin *et al.*, 1981; 1985) and the presence of specific, high affinity binding sites for Tyr-MIF-1 in rat brain has been found (Zadina *et al.*, 1982). In addition, Tyr-MIF-1 has been shown to have antiopiate properties in a number of behavioural paradigms such as morphine-induced antinociception in the tail-flick test and in a chemical test of antinociception also involving morphine administration (Kastin *et al.*, 1984; 1985). These and other demonstrations led us to postulate that Tyr-MIF-1 may be part of an endogenous antiopiate system (Kastin *et al.*, 1979; Galina & Kastin, 1986).

The following experiments were conducted to analyse further the antinociceptive and antiopiate properties of Tyr-MIF-1 in various paradigms of antinociception. These stressors represent different means of activating endogenous antinociceptive systems without the necessity of administering any opiate compounds. Instead, each test was designed to include components of stress-induced analgesia, an endogenous form of analgesia (Watkins & Mayer, 1982; Terman *et al.*, 1984; Galina & Amit, 1986).

Various parameters of shock have been used to induce stress-induced antinociception (Watkins & Mayer, 1982; Terman *et al.*, 1984; Galina & Amit, 1986). As our first stressor, we used 0.5 mA of electric shock administered to all paws of unrestrained mice for 90 s. Preliminary evidence indicated that, in our laboratory, these conditions induce an opiate-mediated increase in pain threshold since the related peptide MIF-1 (Pro-Leu-Gly-NH_2) and naloxone (see Results) antagonized the increased pain thresholds after shock.

The second method of inducing analgesia was the psychological stress of novelty. Novelty is sufficient to raise pain thresholds that can be reduced by the administration of naloxone (Bardo & Hughes, 1979; Advocat, 1983), is associated with increased plasma levels of β-endorphin (Scallet, 1982), and can be affected by opiate tolerance (Bardo & Hughes, 1979; Sherman, 1979; Advocat 1983; Tiffany *et al.*, 1983). The analgesic effects of novelty were measured on the hot-plate whereby a number of different behaviours can be recorded in the same animals (Woolf & MacDonald, 1944). Since the hot-plate also represents a novel environment, it facilitates the determination of the effects of novelty on antinociceptive responses.

Warm-water swimming, the third stressor we used, induces an opiate form of antinociception (Bodnar *et al.*, 1980; O'Connor & Chipkin, 1984). This allowed the assessment of the antiopiate properties of Tyr-MIF-1 in another opiate form of stress-analgesia.

Methods

Male CD-1 mice (Charles River Laboratories, Wilmington, MA) weighing between 22–24 g were used throughout these experiments. They were housed under a 12 h light/dark cycle, 20 per cage, given free access to laboratory chow and water, and were acclimatized to the animal colony for at least 2 weeks before experimentation began. Each experiment was run during the mid-photophase period. A different batch of mice was used for each experiment and each mouse was tested only once.

Tyr-MIF-1 was diluted in 0.9% NaCl solution (saline), 0.01 M acetic acid. In each experiment 4 coded doses were used: 0.0, 0.1, 1, 10.0 mg kg^{-1}. All injections were given i.p. in a volume of 10 ml kg^{-1}. Tyr-MIF-1 was prepared by solid phase synthesis and all material was at least 98.6% pure.

A Columbus Instruments shuttle box (No. 85001–7) was used to administer 90 s of constant current 0.5 mA shock to all four paws. The mice were confined to one side of the shuttle box by means of a plexiglass cylinder (10 × 25 cm).

For the warm-water swim, mice were individually placed within a plexiglass tube (10 × 25 cm) located in a hot-water bath (Precision Scientific, Model No. 82) that maintained the water at 32°C.

The tail-flick apparatus was the same as described previously (Kastin *et al.*, 1984). Immediately before testing, the mice were placed in plexiglass restraining tubes (2.5 × 5 cm). The tail (2 cm from tip) was placed into a groove that housed a nichrome thermal wire that was heated to give a basal latency of 5–6 s. The heat was automatically terminated at 17 s. Removal of the tail resulted in activation of a photoelectric cell that was coupled to an automatic timer. The mean latencies of three consecutive determinations (one min apart) were used for statistical analysis of tail-flick responses.

For the hot-plate test, a commercially available hot-plate (Mod. 35D hot-plate analgesia meter, IITC Inc.) was heated to 55°C (± 0.1°C). A plexiglass tube (10 × 25 cm) confined each mouse to the surface of the plate. Three behavioural measures were recorded: latency to first hind-paw lick, first jump with both back legs in the air, and escape by hanging on the top rim of the cylinder. Mice were tested only once on the hot-plate and were removed if they had not escaped after 90 s.

Shock-induced analgesia

Fifteen min before being placed in the shuttle box, the animals ($n = 10$) were tail-marked, injected with Tyr-MIF-1 or diluent, and replaced in their home cages. After 90 s of shock (0.5 mA, constant current, scrambled), they were tested for antinociception by the tail-flick test as described above. Immediately after the tail-flick test, each mouse was placed in a plastic container (16 × 28 cm). After 5 min in this plastic container, they were tested on the hot-plate once.

Novelty-induced analgesia

Mice ($n = 8$) were injected with Tyr-MIF-1 or diluent and then individually placed in novel plastic boxes (16 × 28 cm) in the unfamiliar room for testing on the hot-plate. Because of the staggered procedure, there were 8 mice in the unfamiliar room while another mouse was being tested. The mice stayed in the unfamiliar room for 15 min and then were placed on the hot-plate for 90 s where behavioural latencies were recorded.

Warm-water swim analgesia

The animals ($n = 8$) were injected Tyr-MIF-1 or diluent and brought to the test room 15 min before being placed into the water-bath. The control groups were dipped (Dip) in the water for 10 s since it has been demonstrated that this is the appropriate control (O'Connor & Chipkin, 1984; Galina & Kastin, 1985). The experimental group was forced to swim for 6 min. Immediately thereafter each mouse was placed in a paper towel-lined container for 1 min after which it was tested on the hot-plate.

Statistical analysis

Analysis of Variance (Anova) followed by post hoc Duncan's Multiple Range Tests ($P < 0.05$) were used throughout.

Results

Shock-induced analgesia

In a preliminary experiment we determined whether our shock parameters induced opiate-mediated responses as measured by naloxone attenuation. We found that these parameters of shock reliably induced a powerful antinociceptive response that was attenuated by naloxone (1 mg kg^{-1}) as measured by paw-lick latencies on the hot-plate test [non-shocked diluent control (14.2 ± 2.2 s), shocked diluent (25.4 ± 4.4 s), non-shocked naloxone (14.7 ± 4.6 s), and shocked naloxone (18.6 ± 2.3 s)]. ANOVA revealed a significant effect of testing condition ($F(1,28) = 34.48$, $P < 0.001$), a significant effect of naloxone ($F(1,28) = 5.86$, $P < 0.02$), and a significant interaction ($F(1,28) = 8.24$, $P < 0.001$) that reflected the activity of naloxone after administration of shock.

Analysis of Variance revelaed that the reduction of

Table 1 The effects of exposure to shock and Tyr-MIF-1 on tail-flick and hot-plate responses

Latencies (s)	Diluent (0.0)	*Dose of Tyr-MIF-1* (mg kg^{-1}) 0.1	1.0	10.0
Tail-flick	14.20	11.87	12.02	12.00
s.e.mean	1.23	3.69	4.93	3.36
Paw-lick	18.45	14.85	10.94*	11.26*
s.e.mean	2.76	2.02	1.22	1.32
Jump	64.46	48.20*	45.50*	43.12*
s.e.mean	4.91	2.52	4.09	4.15
Escape	73.10	55.99*	55.29*	60.16*
s.e.mean	4.03	4.10	4.10	4.30

Asterisks denote significant ($P < 0.05$) differences from Diluent control.

the effect of shock by Tyr-MIF-1 when compared with diluent control in the tail-flick test was not statistically significant. The results for each group are shown in Table 1.

Since no significant result was obtained with the tail-flick test, we decided to use the hot-plate test in this and the next experiments. It is not uncommon to find instances wherein the results of different tests of antinociception are known to yield differential results (Dennis & Melzack, 1980; Holaday & Belenky, 1980). In the hot-plate test (Table 1), the paw-lick ($F(3,36) = 37.48$, $P < 0.05$), jump ($F(3,36) = 161.17$, $P < 0.05$), and escape ($F(3,36) = 201.95$, $P < 0.05$) measures were all significant. Post hoc analysis indicated that the effects of the 1.0 and 10.0 mg doses of Tyr-MIF-1 were different from those of diluent on the paw-lick test. On the jump and escape measures, the effects of all 3 doses of Tyr-MIF-1 were reliably different from those of diluent.

Novelty-induced analgesia

A preliminary experiment indicated that 15 min exposure to the novel environment reliably induced changes in paw-lick latency (uninjected home cage control (17.6 ± 1.2 s) vs uninjected novelty condition (22.0 ± 1.4 s) ($t(16) = -2.58$, $P < 0.05$).

Tyr-MIF-1 significantly reduced the latency to paw-lick ($F(3,31) = 8.22$, $P < 0.001$). Post hoc tests indicated that the effects of the 0.1 and 10.0 mg dose of Tyr-MIF-1 were different from the Diluent group (Table 2). The main effect of Tyr-MIF-1 on the jump

Table 2 The effects of a novel environment and Tyr-MIF-1 on hot-plate responses

Latencies (s)	Diluent (0.0)	*Dose of Tyr-MIF-1* (mg kg^{-1}) 0.1	1.0	10.0
Paw-lick	11.51	6.55*	12.31*	8.61*
s.e.mean	1.06	0.57	1.05	0.95
Jump	79.61	54.16*	53.28*	57.54
s.e.mean	6.16	6.50	9.47	9.47
Escape	71.69	56.70	61.31	64.19
s.e.mean	9.38	5.86	7.65	8.01

Asterisks denote significant ($P < 0.05$) differences from Diluent control.

response did not reach statistical significance ($F(3,31) = 2.43$, $P < 0.08$); however, post hoc tests indicated that the effects of the 0.1 and 1.0 doses were significantly different from Diluent. The tendency of Tyr-MIF-1 to reduce the latency to escape was not statistically significant.

Warm-water swim analgesia

Analysis of Variance was used to analyse the results from only the mice that displayed the paw-lick ($n = 7/8$). There was a significant effect of 6 min swim ($F(1,52) = 59.40$, $P < 0.001$) and dose of Tyr-MIF-1 ($F(3,52) = 6.88$, $P < 0.001$). The results of Duncan's post hoc analyses can be seen in Table 3. That the jump and escape measures were not significicantly different probably reflected the small number of mice responding with these behaviours.

Discussion

The results of these experiments add further support to the evidence that Tyr-MIF-1 can antagonize antinociceptive responses (Kastin *et al.*, 1984; 1985). The results extend these observations to include responses in paradigms designed to assess endogenous analgesic systems not involving the administration of exogenous opiates. Using three different parameters of stress-induced analgesia, we have shown that the antinociception induced by exposure to shock, novelty, or warm-water could all be attenuated by the previous administration of Tyr-MIF-1.

Shock-induced analgesia can be mediated by both opiate and non-opiate factors (Watkins & Mayer, 1982; Terman *et al.*, 1984; Galina & Amit, 1986). Using the antagonist naloxone, the usual method of inferring opiate involvement, we have determined that our conditions induce an opiate-mediated response on the hot-plate (see Results). Also, since antinociception induced by similar conditions as produced here are opiate-mediated responses (Buckett, 1979; Snow & Dewey, 1983), though sometimes strain dependent (Jacob *et al.*, 1986), and since Tyr-MIF-1 antagonized stress-induced antinociception in mice under the conditions of our experiments, it can be assumed that the antinociception produced by our parameters of shock is opiate-mediated.

Tyr-MIF-1 may also function in the same manner as naloxone in reducing the latency after exposure to novelty. The administration of naloxone and Tyr-MIF-1 may sensitize the animal to some antinociceptive measures. Several studies have reported a hyperalgesic effect of naloxone on the hot-plate test using the jump or escape measure but not the paw-lick measure (Jacob *et al.*, 1974; Grevery & Goldstein, 1976; Amir & Amit, 1978; Janicki & Libich, 1979; Holaday & Belenky, 1980; Pilcher, 1980). It is likely that these experiments included components of novelty which induced the activation of an endogenous mechanism that is affected by naloxone or, in our case, Tyr-MIF-1.

With the same conditions of novelty we found a hyperalgesic effect of naloxone. In our laboratory, naloxone (1 mg kg^{-1}) was able to attenuate hot-plate responding even when the paw-lick measure was used (control 9.5 ± 1.5 vs naloxone 6.0 ± 0.7 s $P < 0.05$). Coderre & Rollman (1983) were also able to obtain naloxone-induced hyperalgesia on the hot plate using the paw-lick response and Carmody *et al.* (1979) have found differences on the foot-flick measure after administration of naloxone. The idea that different components of hot-plate responses indicate different mechanisms is not supported by the fact that the effects of opiate agonists are attenuated by naloxone for all responses on the hot-plate or in the tail-flick test which is assumed to measure reflex behaviour. This also fails to support the distinction between sensory/discriminative and motivational/affective components. It is probable that the stress of novelty, which in

Table 3 The effects of a 6 min swim and Tyr-MIF-1 on hot-plate responses

		Dose of Tyr-MIF-1 (mg kg^{-1})		
Latencies (s)	Diluent (0.0)	0.1	1.0	10.0
10 s Dip				
Paw-lick	9.14	6.79*	7.96	8.81
s.e.mean	0.96	0.59	0.57	1.00
6 min Swim				
Paw-lick	14.33	9.99*	14.32	11.31*
s.e.mean	0.81	0.74	0.73	1.21

Asterisks denote significant ($P < 0.05$) differences from Diluent control.

our case includes injection, separation from conspecifics and the smell in the testing environment, is sufficient to induce the release of endogenous peptides that result in elevated pain thresholds. We feel that they might also act by directly rendering the animal more sensitive to the pain-inducing stimulus.

The effects of Tyr-MIF-1 are also similar to those of naloxone after warm-water swimming, a form of analgesia shown to be opiate-mediated (Bodnar *et al.*, 1980; O'Connor & Chipkin, 1984). This once again demonstrates the ability of Tyr-MIF-1 to affect behaviour in the same functional manner as the antiopiate naloxone. MIF-1, a related peptide, also has been found to reduce warm-water swim-induced antinociception (Galina & Kastin, 1985).

This paper adds further support to the concept that MIF-1/Tyr-MIF-1 peptides have opiate antagonist actions (Galina & Kastin, 1985; Kavaliers & Hirst, 1985; Tesky & Kavaliers, 1985) and are part of an antiopiate system that may function to balance the opiate system (Kastin *et al.*, 1979; Galina & Kastin, 1986).

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