COMMUNICATIONS

Effects of Pro-Leu-Gly-NH₂ (MIF) on the antinociceptive and thermoregulatory actions of morphine and oxotremorine

P. SLATER*, S. L. DICKINSON, *Department of Physiology, University of Manchester, Manchester M13 9PT, U.K.*

Some neurohypophyseal hormones, including MIF (Pro-Leu-Gly-NH₂), and analogues modify morphine's pharmacological actions. MIF prevents morphine catalepsy in rats (Chiu & Mishra 1979) and affects the development of tolerance and physical dependence (Van Ree & De Wied 1976). Other central actions of MIF include oxotremorine antagonism (Plotnikoff & Kastin 1975), although this is disputed (Björkman et al 1980). Most work with MIF in rats has involved single doses and it was shown that the acute pretreatment times can influence the results (Turnbull & Wheeler 1980). Recently, it was shown that MIF has a very short half life in rat plasma, although it is remarkably persistent in human plasma (Witter et al 1980). Thus, problems in demonstrating MIF's pharmacological actions in rats may arise because of its rapid metabolism. Recently, we demonstrated that MIF antagonizes morphine's antinociceptive action when given chronically to rats (Dickinson & Slater 1980). We now present more data on chronic MIF and morphine's thermoregulatory and antinociceptive actions. The specificity of MIF's anti-morphine action was investigated by examining its effects on oxotremorine-induced hypothermia and analgesia in mice.

Methods

Male albino mice (25–30 g) and female Sprague Dawley rats (100–120 g) received i.p. injections of MIF (Sigma), 2 mg kg⁻¹, twice daily for 5 days. Control animals were given vehicle 0·9% NaCl (saline) injections.

Hot plate experiments were performed on mice 1 h after the last MIF injection. Antinociceptive activity was measured by recording the time between placing a mouse on a heated metal plate (55 °C) and the response: licking or raising the paws or a determined attempt to escape. A cut-off time of 30 s was used. Three initial trials were performed on control and MIF-treated mice to ensure consistent response times. Hot plate response times were measured every 15 min after i.p. administration of morphine and oxotremorine.

Temperatures of mice housed four to a cage, and

* Correspondence.

rats housed singly, were recorded in a constant temperature (21 °C) room. Measurements were made between 10.00 and 12.00 h, and 1 h after the last dose of MIF, with a thermistor probe inserted in the oesophagus, a procedure which requires brief (20 s) restraint.

Results

The initial hot plate reaction times of saline- and MIF-treated mice were the same (Table 1). Morphine sulphate (5–15 mg kg⁻¹ i.p.) increased the reaction times of both saline- and MIF-treated mice. However, the increases in the reaction times of MIF-treated mice which recieved the larger doses of morphine (10, 15 mg kg⁻¹) were significantly less than the corresponding reaction times of control mice.

Oxotremorine (50, 100 μg kg⁻¹ i.p.) increased the reaction time of mice on the hot plate (Table 2). In contrast to morphine, pretreatment with MIF had no effect of the antinociceptive action of oxotremorine.

MIF had no effect on the oesophageal temperature of rats. Control rats had a temperature of 36·8 ± 0·1 °C (mean ± s.e.m., n = 11) and MIF-treated rats 37·0 ± 0·2 (n = 12). Morphine sulphate (5, 10 mg kg⁻¹ i.p.) caused hyperthermia in both control and MIF-treated rats (Table 3). The rises in temperature recorded in the two groups were not significantly different.

The temperature of control mice was 36·3 ± 0·2 °C (mean ± s.e.m., n = 17) and that of mice treated

Table 1. Effect of MIF on the antinociceptive action of morphine in mice.

Pretreatment[a]	Morphine mg kg⁻¹ i.p.	Mean reaction time Normal	Mean reaction time Post-morphine, 30 min
Saline	5	8·9±0·4	13·6±1·0[b]
MIF	5	9·4±0·2	11·8±0·7[b]
Saline	10	8·7±0·1	19·0±0·7[b]
MIF	10	7·1±0·4	14·5±0·2[bc]
Saline	15	10·0±0·2	24·8±0·5[b]
MIF	15	10·4±0·2	20·9±0·4[bc]

[a] MIF 2 mg kg⁻¹ i.p., twice daily, 5 days.
[b] Significantly greater than corresponding normal (*t*-test) $P < 0·05$.
[c] Significantly different from corresponding saline group $P < 0·05$.

Table 2. Effect of MIF on the antinociceptive action of oxotremorine in mice.

Pre-treatment[a]	Oxo-tremorine μg kg^{-1} i.p.	Mean reaction time (s $\pm$ s.e.m., n $=$ 9)	
		Normal	Post-oxotremorine, 45 min
Saline	50	6.0 ± 0.6	10.3 ± 2.5^b
MIF	50	5.7 ± 0.6	8.5 ± 1.0^b
Saline	100	5.1 ± 0.9	21.9 ± 1.7^b
MIF	100	5.3 ± 0.7	20.0 ± 2.5^b

[a] MIF 2 mg kg^{-1} i.p., twice daily, 5 days.
[b] Significantly greater than corresponding normal (t-test) $P < 0.05$.

chronically with MIF was 36.2 ± 0.1 °C (n $=$ 18). Morphine sulphate (5–15 mg kg^{-1} i.p.) caused a dose-related fall in the oesophageal temperature of control mice. MIF treatment had no significant effect on the hypothermia produced by 5–10 mg kg^{-1} of morphine, but partly antagonized the effect of 15 mg kg^{-1} (Table 3).

Oxotremorine (50, 100 μg kg^{-1}) caused a profound fall in the temperature of mice. Pretreatment with MIF had no effect on the hypothermic response to oxotremorine (Table 4).

Discussion
The results, together with previous findings (Kastin et al 1979; Dickinson & Slater 1980), demonstrate that

MIF has weak anti-opiate actions in vivo, provided that an appropriate dose schedule is used. The fact that MIF antagonized the antinociceptive action of morphine, but not oxotremorine, demonstrates specificity for opiate receptor mediated events, because oxotremorine has no effect on opiate receptors (Ireson 1970; Pleuvry & Tobias 1971). The presence of multiple opiate receptors in the c.n.s. has been postulated (Chang & Cuatrecasas 1979) and in vitro studies have shown that MIF antagonizes responses produced by μ-type opiate receptors (Dickinson & Slater 1980).

Thermoregulation is a complex physiological process involving several neurotransmitters (Bligh 1979). Morphine usually has opposite effects on the temperature of mice and rats (Lotti 1973). This has been confirmed in the present study, and although the cause is largely unknown it may reflect a relatively unexplored species difference related to brain monoamines and temperature control (Slater & Blundell 1980). The present results have shown that MIF partly prevented morphine-induced temperature changes in the mouse but not in the rat. Furthermore, the weak antagonism of hypothermia in mice provided another example of a specific anti-opiate action of MIF, because oxotremorine hypothermia was not affected.

This study demonstrates that, at the doses used, MIF weakly and selectively antagonizes the analgesic and hypothermic actions of morphine.

Table 3. Effect of MIF pretreatment on temperature changes produced by morphine in rats and mice.

Species	Pretreatment[a]	Morphine mg kg^{-1} i.p.	n	Temperature changes (mean °C $\pm$ s.e.m.)					
				15 min	30 min	45 min	60 min	90 min	180 min
Rat	Saline	5	6	$+0.3\pm0.02$	$+0.6\pm0.01$	—	$+1.3\pm0.1$	$+1.1\pm0.2$	$+0.6\pm0.1$
	MIF	5	6	$+0.6\pm0.03$	$+1.1\pm0.06$	—	$+1.4\pm0.04$	$+1.4\pm0.3$	$+0.5\pm0.1$
	Saline	10	6	$+0.6\pm0.03$	$+0.9\pm0.2$	—	$+1.6\pm0.2$	$+1.6\pm0.4$	$+1.4\pm0.2$
	MIF	10	6	$+0.5\pm0.01$	$+0.7\pm0.3$	—	$+1.2\pm0.4$	$+1.8\pm0.4$	$+1.3\pm0.2$
Mouse	Saline	5	6	-0.7 ± 0.2	—	-1.0 ± 0.1	-0.3 ± 0.1	—	—
	MIF	5	7	-1.0 ± 0.3	—	-1.3 ± 0.3	-0.6 ± 0.2	—	—
	Saline	10	7	-2.3 ± 0.1	—	-1.9 ± 0.1	-1.7 ± 0.1	—	—
	MIF	10	6	-2.4 ± 0.04	—	-2.2 ± 0.2	-2.1 ± 0.1	—	—
	Saline	15	6	-3.0 ± 0.05	—	-3.7 ± 0.1	-2.9 ± 0.02	—	—
	MIF	15	8	-2.3 ± 0.1^b	—	-2.8 ± 0.04^b	-2.0 ± 0.01^b	—	—

[a] MIF 2 mg kg^{-1} i.p., twice daily, 5 days.
[b] Significantly different from corresponding control (t-test) $P < 0.05$.

Table 4. Effect of MIF pretreatment on hypothermia produced by oxotremorine in mice.

Pretreatment[a]	Oxotremorine μg kg^{-1} i.p.	n	Temperature changes (mean °C $\pm$ s.e.m.)						
			15 min	30 min	45 min	60 min	90 min	120 min	150 min
Saline	50	6	-3.1 ± 0.3	-6.1 ± 0.6	-6.1 ± 0.7	-5.1 ± 0.2	-3.0 ± 0.3	-1.0 ± 0.1	$+0.1\pm0.02$
MIF	50	6	-2.6 ± 0.2	-6.8 ± 0.4	-5.9 ± 0.4	-4.8 ± 0.1	-1.9 ± 0.1	-1.1 ± 0.1	-0.2 ± 0.02
Saline	100	8	-4.7 ± 0.1	-5.8 ± 0.4	-6.6 ± 0.3	-6.7 ± 0.2	-5.1 ± 0.1	-2.5 ± 0.1	-0.5 ± 0.01
MIF	100	8	-4.8 ± 0.1	-7.2 ± 0.7	-8.2 ± 0.9	-7.8 ± 0.9	-4.8 ± 0.6	-3.1 ± 0.3	$-1.4\pm.004$

[a] MIF 2 mg kg^{-1} i.p., twice daily, 5 days.

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Determination of biological activity of alprazolam, triazolam and their metabolites

VIMALA H. SETHY*, DOUGLAS W. HARRIS, The Upjohn Company, CNS Research, Kalamazoo, Michigan 49001, U.S.A.

The benzodiazepines are psychoactive drugs with wide therapeutic applications as anxiolytics, anticonvulsants, and muscle relaxants (Zbinden & Randall 1967). Their site and mechanism of action have recently become the subject of extensive investigations because of the availability of tritium-labelled diazepam and flunitrazepam with high specific activity. Using these ligands, in vitro and in vivo binding studies have indicated that a specific high affinity binding site, the 'benzodiazepine receptor', in the brain may be relevant to the pharmacological action of the drugs (Mohler & Okada 1977; Squires & Braestrup 1977; Tallman et al 1979).

Triazolobenzodiazepines, like alprazolam and triazolam, have been found to be both anxiolytic and hypnotic in man (Fabre & McLendon 1979; Chatwin & John 1976) in whom these drugs are metabolized to hydroxylated compounds and benzophenones, which are excreted in urine (Eberts et al 1980, 1981). The activity of alprazolam, triazolam and their metabolites on the benzodiazepine receptor is not known. We have undertaken to determine the activity of these compounds on benzodiazepine receptors by using [3H]flunitrazepam ([3H]FNZ) binding to crude rat brain membrane preparations. The results of in vitro [3H]FNZ binding assays were compared with the in vivo ED50s of these compounds required to protect the mice against leptazol(pentetrazol)- and nicotine-induced seizures and against hypoxic stress.

Benzodiazepine receptor binding assays were carried out according to Speth et al (1979). Male Sprague-Dawley rats (180–200 g) were decapitated, their brains quickly removed and, minus cerebellum, homogenized in 10 volumes of cold (4 °C) distilled water, using a Brinkman Polytron PCU-110 homogenizer for 30 s at setting No 6, and centrifuged at

* Correspondence.

48 000 g at 4 °C for 10 min. The pellet was washed three times by resuspension and recentrifugation, as described above. The final pellet was then suspended in 50 volumes of distilled water.

[3H]FNZ binding was measured by incubating 1·0 ml aliquots of membrane suspension with 0·1 ml of [3H]FNZ (specific activity 87·9 Ci mmol^{-1}) to give a final concentration of 0·7 nM for IC50 determinations (or in the range of 0·1–3·2 nM for Scatchard analysis), 0·1 ml of water or drug, as indicated, and 0·8 ml of phosphate-buffered saline (81 mM, Na_2HPO_4, 9·5 mM KH_2PO_4, and 100 mM sodium chloride), pH 7·4, to give a final volume of 2 ml. The mixture was incubated for 100 min at 0–4 °C and then filtered under vacuum through a Whatman GF/B filter. The incubation tube was rinsed with 5 ml of ice cold buffered saline and this rinse was applied to the filter. The filter was finally washed three times with 5 ml amounts of buffered saline. The filter paper was placed in a scintillation vial containing 15 ml of Amersham Searle ACS^R cocktail and the radioactivity was counted by a liquid scintillation spectrometer.

Specific binding was defined as the total binding minus binding in the presence of 100 μM flurazepam. Specific binding represented over 90% of the total binding. Data were subjected to Scatchard and Hill analyses to determine the dissociation constant (K_d), maximum number of binding sites (B_{max}), and Hill coefficient. The IC50 was obtained by logit-log plot of the data. Inhibition constant (K_i) was calculated by the following equation: IC50/1 + (c/K_d), where c = concentration of ligand (0·7 nM) and K_d = dissociation constant (1·5 nM). The ED50 dose for protection of mice against leptazol- and nicotine-induced seizures and against hypoxic stress was determined by the method of Gall et al (1978) and Moffett et al (1976).

The high affinity specific binding of [3H]FNZ was