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Comparative Effects of Prolyl-Leucyl-Glycinamide and Naloxone on Morphine-Induced Inhibition of Gastrointestinal Transit

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PILLAI, N. P. AND H. N. BHARGAVA. *Comparative effects of prolyl-leucyl-glycinamide and naloxone on morphine-induced inhibition of gastrointestinal transit.* PHARMACOL BIOCHEM BEHAV **21**(3) 365–368, 1984.— The effects of prolyl-leucyl-glycinamide (MIF) and naloxone on the gastrointestinal transit in mice were investigated using the charcoal meal test. MIF administered intraperitoneally (IP) (1–10 mg/kg) or intracerebroventricularly (ICV) (10 μg/mouse) had no effect on the transit. Administration of morphine by subcutaneous (SC) route significantly inhibited the gastrointestinal transit. The morphine-induced inhibition of the transit was not affected by MIF whether given by IP or ICV route. Administration of the opiate antagonist naloxone (1 mg/kg, IP or 10 μg/mouse, ICV) had no effect on the gastrointestinal transit, but it significantly antagonized the inhibition produced by morphine. Some earlier studies have indicated narcotic antagonistic effect of MIF. However, in the present study, evidence for such an action of MIF was not obtained. It is suggested that MIF does not appear to have narcotic antagonistic activity and further supports an earlier study from this laboratory that MIF may not interact with opiate receptors.

Morphine MIF Naloxone Gastrointestinal transit Narcotic antagonism

IT is well known that the release of melanocyte stimulating hormone from the anterior pituitary is controlled by the hypothalamic tripeptide, melanotropin release inhibiting factor (MIF, prolyl-leucyl-glycinamide) [11]. In addition to the endocrine function, MIF also has non-endocrinological actions. For example, it interacts with the dopaminergic, cholinergic and opiate receptor systems. It has been reported that MIF potentiates the behavioral effects of the dopaminergic agonist l-dopa [14] and antagonizes the peripheral and central effects of the cholinergic agonist oxotremorine in mice [13]. MIF has been shown to have varying effects on the opiate receptor system. Kastin *et al.* [10] reported that acute administration of MIF inhibited the analgesic effect of morphine and enkephalins in mice. However, Chiu and Mishra [7] could not find such an effect of MIF in rats even though they found that MIF inhibited morphine-induced catalepsy in these animals. On the other hand, Dickinson and Slater [9] found that acute administration of MIF had no effect on the analgesic effect of morphine in mice while chronic treatment significantly antagonized morphine-induced analgesia in rats but it had only a weak antagonistic activity in mice. Moreover, they found that chronic treatment with MIF reduced the morphine-induced initial phase of depression of locomotor activity in mice while it potentiated the stimulant phase of locomotor activity.

MIF and its derivatives have also been shown to inhibit the development of tolerance to and dependence on opiates in mice and rats [1, 2, 3, 4, 17] without affecting the analgesic effect of acute morphine administration [5].

Since some of the earlier investigations suggested opiate antagonistic activity for MIF [7, 9, 10], the possibility for such an action was tested in the present study by investigating the effect of MIF on the morphine-induced reduction of gastrointestinal transit in mice.

METHOD

Male Swiss Webster mice weighing 20–25 g were used. They were housed for at least four days prior to being used in a room with controlled temperature (23±1°C), humidity (65±2%) and light (L 0600–1800 hr). Food was withdrawn 24 hr before the experiment but water was allowed ad lib.

Assessment of Gastrointestinal Transit

The gastrointestinal transit was assessed by charcoal meal test. The animals were given 0.3 ml of a suspension of charcoal (10% charcoal in 5% gum acacia) intragastrically. After 20 min, they were sacrificed by cervical dislocation. The abdomen was cut open and the intestine from the pyloric end to the ileocaecal junction was dissected out. The dis-

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TABLE 1

EFFECT OF MIF ON THE GASTROINTESTINAL TRANSIT IN MICE

Treatment*	Dose	Gastrointestinal transit (%) Mean ± S.E.M. (n=8)
	Experiment 1	
Vehicle	10 ml/kg, IP	52.4 ± 1.8
MIF	1 mg/kg, IP	50.9 ± 1.9
MIF	3 mg/kg, IP	59.6 ± 4.0
MIF	10 mg/kg, IP	48.9 ± 2.8
	Experiment 2	
Vehicle	5 μl, ICV	67.8 ± 2.0
MIF	10 μg, ICV	65.6 ± 2.3

*MIF was given 10 min (IP) prior to or immediately (ICV) prior to charcoal meal. Experiments 1 and 2 were done on two different days.

TABLE 2

EFFECT OF IP ADMINISTRATION OF MIF ON THE MORPHINE-INDUCED REDUCTION IN GASTROINTESTINAL TRANSIT IN MICE

Treatment*	Gastrointestinal transit (%) Mean ± S.E.M. (n=8)
Vehicle (IP) + Vehicle (SC)	55.5 ± 2.0
Vehicle (IP) + Morphine (5 mg/kg SC)	17.2 ± 3.0†
MIF (1 mg/kg, IP) + Morphine (5 mg/kg, SC)	16.0 ± 1.0†
MIF (3 mg/kg, IP) + Morphine (5 mg/kg, SC)	20.0 ± 1.8†
MIF (10 mg/kg, IP) + Morphine (5 mg/kg, SC)	17.6 ± 2.4†

*MIF and morphine were administered 10 min prior to the charcoal meal.
†$p<0.001$ compared to vehicle (IP) + vehicle (SC).

TABLE 3

EFFECT OF ICV ADMINISTRATION OF MIF ON THE MORPHINE-INDUCED REDUCTION IN GASTROINTESTINAL TRANSIT IN MICE

Treatment*	Gastrointestinal transit (%) Mean ± S.E.M. (n=8)
Vehicle (ICV) + Vehicle (SC)	69.0 ± 1.8
MIF (10 μg, ICV) + Vehicle (SC)	64.4 ± 2.5
Vehicle (ICV) + Morphine (5 mg/kg, SC)	29.0 ± 2.9†
MIF (10 μg, ICV) + Morphine (5 mg/kg, SC)	23.0 ± 3.7†

*MIF and morphine were given immediately prior to the charcoal meal.
†$p<0.001$ compared to vehicle (ICV) + vehicle (SC).

TABLE 4

EFFECT OF IP ADMINISTRATION OF NALOXONE ON THE MORPHINE-INDUCED REDUCTION IN GASTROINTESTINAL TRANSIT IN MICE

Treatment*	Gastrointestinal transit (%) Mean ± S.E.M. (n=8)
Vehicle (IP) + Vehicle (SC)	54.0 ± 3.4
Naloxone (1 mg/kg, IP) + Vehicle (SC)	56.2 ± 2.4
Vehicle (IP) + Morphine (5 mg/kg, SC)	22.9 ± 1.1†
Naloxone (1 mg/kg, IP) + Morphine (5 mg/kg, SC)	39.9 ± 2.2†‡

*Naloxone and morphine were given 10 min prior to the charcoal meal.
†$p<0.001$ compared to vehicle (IP) + vehicle (SC).
‡$p<0.001$ compared to vehicle (IP) + morphine (SC).

TABLE 5

EFFECT OF ICV ADMINISTRATION OF NALOXONE ON THE MORPHINE-INDUCED REDUCTION IN GASTROINTESTINAL TRANSIT IN MICE

Treatment*	Gastrointestinal transit (%) Mean ± S.E.M. (n=8)
Vehicle (ICV) + Vehicle (SC)	69.0 ± 1.8
Naloxone (10 μg, ICV) + Vehicle (SC)	63.9 ± 4.0
Vehicle (ICV) + Morphine (5 mg/kg, SC)	29.0 ± 2.9†
Naloxone (10 μg, ICV) + Morphine (5 mg/kg, SC)	46.4 ± 4.4‡†

*Naloxone and morphine were given immediately prior to the characoal meal.
†$p<0.001$ compared to vehicle (ICV) + vehicle (SC).
‡$p<0.01$ compared to vehicle (ICV) + morphine (SC).

tance traveled by charcoal and the length of the intestine were measured. The gastrointestinal transit was expressed as the percentage of the distance traveled by charcoal out of the total length of the intestine.

Effect of MIF on the Gastrointestinal Transit

Different doses of MIF (1, 3 and 10 mg/kg) dissolved in saline were given intraperitoneally (IP) 10 min before the charcoal meal and the gastrointestinal transit was measured. Control animals received saline (10 ml/kg, IP). MIF was also given by intracerebroventricular (ICV) route (10 μg) following the method described earlier [12]. Control animals received an equal volume of the vehicle (5 μl/mouse). The charcoal meal was given immediately after the ICV administration of MIF or saline.

Effect of MIF in Combination with Morphine on the Gastrointestinal Transit

The effect of MIF on morphine-induced decrease in gastrointestinal transit was investigated by administering MIF (1,3 and 10 mg/kg) IP along with a subcutaneous (SC) injection of morphine sulphate (dissolved in saline, 5 mg/kg). Control animals received vehicle treatment. The animals were given charcoal meal 10 min after the administration of MIF and morphine. In some experiments, MIF was also given by the central route (10 μg/mouse ICV) along with morphine (5 mg/kg, SC) just prior to the charcoal meal.

Effect of Naloxone on the Inhibitory Effect of Morphine on the Gastrointestinal Transit

The effect of peripheral (IP) as well as central (ICV) administration of naloxone on the morphine-induced reduction of gastrointestinal transit was also investigated in the present study. Naloxone hydrochloride (1 mg/kg, IP or 10 μg/mouse, ICV) or saline was administered along with morphine (5 mg/kg, SC) or saline and the charcoal meal was given 10 min after the IP administration or immediately after the ICV administration of MIF.

RESULTS

Effect of MIF on the Gastrointestinal Transit

Administration of 3 different doses (1,3 and 10 mg/kg) of MIF by IP route did not significantly alter the gastrointestinal transit of mice as assessed by the charcoal meal test (Table 1). Even the administration of MIF by the central route (ICV) at a dose of 10 μg/mouse failed to alter the motility of gastrointestinal tract.

Effect of MIF in Combination with Morphine on the Gastrointestinal Transit

Administration of morphine (5 mg/kg, SC) produced a significant inhibition of the gastrointestinal transit (Table 2). When morphine was combined with different doses of MIF (1,3 and 10 mg/kg) by IP route, MIF did not antagonize the effect of morphine since the inhibitory effect of morphine on the gastrointestinal motility remained unaltered (Table 2). Moreover, administration of MIF (10 μg) by the ICV route also did not significantly affect the inhibitory effect of morphine (Table 3).

Effect of Naloxone on the Inhibitory Effect of Morphine on the Gastrointestinal Transit

The IP administration of the opiate antagonist naloxone did not produce any significant effect on the gastrointestinal transit (Table 4). However, when administered along with morphine, the opiate antagonist significantly antagonized the inhibitory effect of morphine. The effect observed after IP administration was also noticed when naloxone was given by the ICV route. At a dose (10 μg/mouse, ICV) which by itself did not produce any effect on the gastrointestinal motility, naloxone antagonized the effect of morphine on the gastrointestinal tract (Table 5).

DISCUSSION

Some of the earlier investigations have suggested that MIF could act as an opiate antagonist as revealed by the antagonism of a few actions of morphine and enkephalins by MIF [7, 9, 10]. However, the present study reveals that MIF does not have any opiate antagonistic activity on the gastrointestinal motility.

It has been reported that the inhibitory effect of morphine on the gastrointestinal motility is mediated by peripheral, as well as, central mechanisms [6,15]. In the present study, the opiate antagonist, naloxone given by peripheral or central route significantly antagonized the effect of morphine thus confirming the involvement of opiate receptors in mediating the actions of morphine on the gastrointestinal tract and also that the gastrointestinal transit in mice as assessed by charcoal meal test can be used as a reliable parameter to evaluate opiate agonists and antagonists. Since in the present study, the central as well as, peripheral administration of MIF failed to antagonize the effect of morphine on the gastrointestinal transit, it can be suggested that MIF does not appear to possess any opiate antagonistic activity. It is also worth mentioning that the antagonism of opiate analgesia by MIF as reported by Kastin *et al.* [10] could not be confirmed in other laboratories [7,8]. Moreover, the present findings also support an earlier report from this laboratory [5] which indicated that MIF neither alters morphine-induced analgesia nor does it appear to affect the binding of radioligands for μ, δ, and κ opiate receptors to the brain membranes.

The failure of MIF to alter the effect of morphine can not be attributed to its short half-life in plasma (approximately 9 min) [16] since in the present study, MIF was given concommitantly (IP as well as ICV) with morphine.

In conclusion, the present study indicates that MIF does not appear to have any opiate antagonistic activity and supports an earlier report from this laboratory [5]. Further studies are in progress to confirm these findings.

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