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MIF (Pro-Leu-Gly-NH_2): failure to affect oxotremorine effects in mice and rats as well as fluphenazine catalepsy or amphetamine hyperactivity in rats

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Oxotremorine antagonism by the tripeptide prolylleucylglycineamide, MIF, was first reported by Plotnikoff et al (1972). All evaluated symptoms of oxotremorine in mice, viz tremor, head twitch, ataxia, lachrymation, salivation and diarrhoea, were attenuated or abolished by MIF, 1–16 mg kg^{-1}. The peptide was active after s.c., i.p., i.v. or oral administration (Plotnikoff & Kastin 1974). These results were partially confirmed in studies where only the tremor-inhibiting effect was evaluated by means of electronic tremor measurement (Castensson et al 1974; Björkman & Sievertsson 1977). In contrast, Kruse (1977) reported a failure of MIF to antagonize oxotremorine tremor in mice. In view of the conflicting results reported for this effect of MIF and also for its influence on opiate tolerance and dependence (Mucha & Kalant 1979) we wish to report an observed lack of effect of MIF in oxotremorine-treated mice and rats as well as in fluphenazine-treated (cf. Voith 1977) or amphetamine-treated rats.

Oxotremorine-treated mice. Male NMRI mice (22–26 g) were given 0·9% NaCl (saline), 3, 10 or 30 mg kg^{-1} MIF s.c. and, 1 h later, oxotremorine sesquioxalate 0·4 mg kg^{-1} (as free base) *s.c.* (6 mice in each group). Their body temperature was recorded every 20 min by means of a thermistor probe and the symptoms enumerated by Plotnikoff et al 1974 were estimated by an experienced observer who was unaware of drug treatment. The mice were monitored for 4 h. The effects of oxotremorine were as earlier reported with the exception that no head twitches were observed. MIF, 10 mg kg^{-1}, seemed to cause a transient reduction of tremor. However, statistical significance was not achieved (Fig. 1). MIF had no appreciable effect on the other symptoms caused by oxotremorine, i.e. hypokinesia, rigidity, hypothermia, lachrymation, salivation and diarrhoea. MIF itself, 10 mg kg^{-1} s.c., had no effect on body temperature.

Oxotremorine-treated rats. Male Sprague-Dawley rats (230–250 g) in an analogous experiment were treated with saline, 0·3, 1, 3, 10 or 30 mg kg^{-1} MIF s.c. followed by oxotremorine (sesquioxalate) 0·4 mg kg^{-1} s.c. (3 rats in each group). The intense whole-body tremor caused by oxotremorine in mice was not observed in the rats, but the tremor was visible in the forepaws and the muscles of the jaws at 20 min, fading to palpable tremor of the jaws at 40–60 min with no response at 100 min. Strong head twitches were also observed. MIF had no effect either on these symptoms or on the oxotremorine-induced hypothermia, rigidity, lachrymation, salivation and diarrhoea.

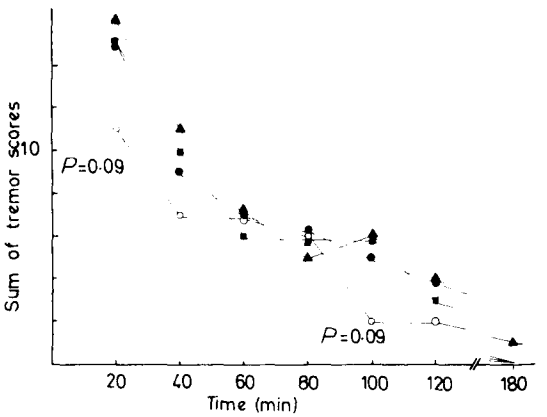

FIG. 1. Oxotremorine tremor, expressed as sums of the individual tremor scores in each group of 6 mice, at various doses of MIF and times after oxotremorine injection. The tremor was scored as: 3 = continuous tremor, 2 = intermittent tremor and 1 = tremor elicited by touch (Cho & Jenden 1964). The *P* values refer to MIF, 10 mg kg^{-1}, vs saline (Mann-Whitney U test; Siegel 1956).

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Amphetamine-treated rats. Male Sprague-Dawley rats (220–260 g), individually caged ($n = 6$), were pretreated with saline or MIF, 50 mg kg^{-1} s.c., for five consecutive days. On day 5 the animals were given (+)-amphetamine sulphate, 4 mg kg^{-1} (as free base) s.c., and their behaviour was evaluated every half hour for four hours. The symptoms induced by the amphetamine included rearing, locomotion, sniffing, head swaying and piloerection. No differences were observed between saline- and MIF-pretreated animals. A rating scale adapted by Widerlöv & Lewander (1978) was used. Over 30–150 min, the scores did not vary by more than 1 from the median (6–7) at each time and at 210 and 240 min the range was 1–3 around median scores of 4–1.

Fluphenazine-treated rats. Male Sprague-Dawley rats (220–260 g) were pretreated with saline or MIF, 50 mg kg^{-1} s.c., for five consecutive days. On day 5 the animals were given fluphenazine HCl (Dapotum), 10 mg kg^{-1} orally or 5 mg kg^{-1} s.c., and the catalepsy was scored over 30–360 min by a 'blind' observer according to stages I–IV of Wirth et al (1958) to give a maximum score/rat of 8. No differences were observed between saline- and MIF-pretreated animals (The results for both groups ($n = 6$) were medians 6–8 (ranges 1–8) at 10 mg kg^{-1} oral or 5–8 (ranges 2–8) at 5 mg kg^{-1} s.c. fluphenazine).

The MIF used was a gift from Abbott Inc. and proved identical to several batches of MIF synthesized in this laboratory (Castensson et al 1974). The chemical stability of the peptide is high (Brtnik et al 1975).

To our knowledge, MIF has been tested as an oxotremorine antagonist in four different laboratories and on four different mouse strains. These are: Abbott laboratories; male ICR and Charles River hypophysectomized (Plotnikoff et al 1972; Plotnikoff & Kastin 1974), University of California, L. A.; male Swiss-Webster (Castensson et al 1974), Hoechst Laboratories; NMRI (Kruse 1977) and University of Uppsala; male NMRI (Björkman & Sievertsson 1977). Oxotremorine antagonism by MIF was in the first case observed by visual scoring and in the second by electronic tremor measurement. Kruse briefly reported negative results. Our present observational study failed to reveal a significant oxotremorine antagonism, but in a modified model (Björkman & Sievertsson 1977), with the aid of an electronic transducer, a slight but significant tremor inhibition could be discerned. Large inter-strain differences as regards the reactions of mice to oxotremorine have been reported (Lush & Andrews 1978). Corresponding differences are conceivable as regards the effects of MIF and other peptides.

Plotnikoff et al (1974a) reported that MIF potentiates the behavioural actions of dopa in pargyline-treated rats (Sprague-Dawley). Using the same rat strain, we were unable to ascertain any oxotremorine antagonism.

The lack of effect of MIF on amphetamine-treated rats is in agreement with the findings of Sandman & Kastin (1978).

The trials with fluphenazine were designed to duplicate the experiments reported by Voith (1977), who also used Sprague-Dawley rats. In contrast to his findings, chronic treatment with MIF, 50 mg kg s.c., did not influence the fluphenazine-induced catalepsy. No explanation for this discrepancy can be offered at present.

In conclusion, we have been unable to fully reproduce previously reported effects of MIF. Unless strain differences or marginal differences in procedure are invoked as explanations for these discrepancies, the claimed pharmacological effects of MIF seem uncertain. Obviously, additional comparative studies are indicated.

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