Oxotremorine Antagonism by a Hypothalamic Hormone, Melanocyte-Stimulating Hormone Release-Inhibiting Factor (MIF) (36558)

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Recently, we reported data indicating that MSH release-inhibiting factor (MIF) potentiates the behavioral effects of DOPA both in normal and hypophysectomized mice (1). The experiments in that report which were performed with hypophysectomized mice established that MIF exerts an action upon the central nervous system (CNS) that is independent of its MSH release-inhibiting activity. This was the first "extrapituitary" effect to be shown for any hypothalamic hormone.

The effects of tremorine in inducing tremor in animals were originally described by Everett (2). Subsequently, oxotremorine was shown to be the active metabolite responsible for these actions (2) and has been widely used to investigate drugs for treatment of Parkinson's disease. Since DOPA has been shown by Everett to antagonize the tremors caused by oxotremorine (4), and since MIF potentiates some CNS effects of DOPA (1), we decided to investigate the effects of MIF in mice using the same system involving oxotremorine.

Methods. Normal (ICR) or hypophysectomized (CD-1, Charles River) male mice (16–20 g) were pretreated with varying doses of MIF (L-Pro-L-Leu-Gly-NH2) (5) one and 4 hr before administration of oxotremorine (0.5 mg/kg, ip). Eight mice were tested at each of six doses of MIF in the intact and hypophysectomized mice. Their responses were recorded by observation techniques (6) and were compared with those observed in 16 intact and 16 hypophysectomized mice which received only oxotremorine as a control.

In studies designed to test the anticholinergic effects of MIF, segments of terminal ileum from adult, male guinea pigs (Scientific Labs) were suspended in a 30 ml chamber, bathed in Tyrode solution at 37°, and aerated with 95% oxygen–5% CO_2. Test compounds were added directly to the tissue chamber 3 min before the addition of an amount of acetylcholine found to produce submaximal contractions. Inhibition of acetylcholine was recorded as the percentage decrease in amplitude as compared with the spasm before treatment.

Results. Control animals receiving oxotremorine exhibited marked signs of parasympathetic stimulation consisting of tremors, head twitch, decreased activity, ataxia, lachrymation, salivation, and diarrhea. MIF, in a dose range of 2 to 16 mg/kg, reduced the tremor induced by oxotremorine in normal mice and blocked the peripheral effects as well. The degree of this antagonism did not seem to differ between the 1 and 4 hr test periods (Table I). MIF was also observed to potentiate the effects of L-DOPA in reducing the central and peripheral effects of oxotremorine. Thus, a minimally active dose of L-DOPA (100 mg/kg ip) plus MIF (0.25 to 1 mg/kg) provided significant protection against the effects of oxotremorine (Table II).

In hypophysectomized mice, oxotremorine was just as effective as in intact mice. In each group of animals, all the expected CNS and peripheral effects were seen. This finding extends the unpublished observations of one of us (AJK) made several years ago that

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TABLE I. Oxotremorine Antagonism Effects of MIF in Normal Intact Mice.[a]

Ip route (mg/kg)	Tremors	Head twitch	Ataxia	Lachry-mation	Sali-vation	Diarrhea
Oxotremorine alone, 0.5 mg/kg	3[b]	3	2	2	3	3
1 hr test						
MIF + oxotremorine						
0.5	3	3	2	2	3	3
1	2	2	2	2	2	2
2	2	2	2	1	2	1
4	2	1	1	1	1	1
8	2	1	1	0	1	1
16	1	1	1	0	1	1
4 hr test						
MIF + oxotremorine						
0.5	3	3	2	2	3	3
1	3	3	2	1	2	2
2	2	2	2	1	2	1
4	2	2	2	1	2	1
8	2	1	1	1	2	1
16	1	1	1	0	1	1

[a] Eight mice per dose.
[b] Degree of oxotremorine effects: 3 = marked; 2 = moderate; 1 = slight; 0 = none.

oxotremorine is effective in hypophysectomized albino rats. MIF was found to be as active in reducing the effects of oxotremorine in hypophysectomized mice as in intact mice (Table III).

No significant anticholinergic activity was observed with MIF when concentrations of 5, 10, and 50 μg/ml were added to the isolated guinea pig ileum. At the high concentration of 100 μg/ml, however, there appeared to be some inhibition of the spasm induced by acetylcholine (Table IV).

Discussion. Our previous study indicated that MIF potentitates the behavioral effects of DOPA (1). The present study shows that MIF also antagonizes the CNS and peripheral effects of oxotremorine. In both investigations, MIF was just as active in hypophysec-

TABLE II. Oxotremorine Antagonism by MIF and L-DOPA in Mice.

	Tremors	Head twitch	Ataxia	Lachry-mation	Sali-vation	Diarrhea
Oxotremorine alone	3[a]	2	2	2	3	3
Ip route L-DOPA alone (mg/kg)						
100	2	2	2	1	2	2
200	1	1	1 (increased motor activity)	1	1	0
400	0	1	0 (increased motor activity)	0	0	0
MIF + L-DOPA (mg/kg)						
0.1 + 100	2	1	1	1	2	0
0.25 + 100	1	1	0	0	1	0
0.5 + 100	1	1	0	0	1	0
1.0 + 100	1	1	0	0	1	0

[a] Degree of oxotremorine effects: 3 = marked; 2 = moderate; 1 = slight; 0 = none.

TABLE III. Oxotremorine Antagonism Effects of MIF in Hypophysectomized Mice.[a]

Ip route (mg/kg)	Tremors	Head twitch	Ataxia	Lachry-mation	Sali-vation	Diarrhea
Oxotremorine alone 0.5 mg/kg	3[b]	3	2	2	3	3
1 hr test						
MIF + oxotremorine						
0.5	3	3	2	2	3	3
1	2	2	2	2	2	2
2	2	2	2	1	2	1
4	2	1	1	1	1	1
8	2	1	1	0	1	0
16	1	1	1	0	0	0
4 hr test						
MIF + oxotremorine						
0.5	3	3	2	2	3	3
1	3	3	2	1	2	2
2	2	2	2	1	2	2
4	2	2	2	1	2	1
8	2	2	1	1	2	1
16	1	1	1	0	0	0

[a] Eight mice per dose.

[b] Degree of oxotremorine effects: 3 = marked; 2 = moderate; 1 = slight; 0 = none.

tomized as in intact mice. These studies clearly demonstrate that MIF exerts a central action that is independent of its action on the pituitary.

Since oxotremorine has been reported to lower brain dopamine/norepinephrine levels in rodents (6), one might speculate whether the antagonistic effects of MIF may involve dopaminergic systems. Earlier studies by Everett, Morse and Borcherding (4), indicated that DOPA also antagonized the effects of oxotremorine. Perhaps MIF accelerates the turnover rates of dopamine in the brain.

The effects of oxotremorine in mice have been considered by many investigators to resemble the symptoms of Parkinson's disease. DOPA has been widely used in the treatment of parkinsonism. In mice, MIF affects the actions of both oxotremorine and DOPA. These findings suggest the need to carefully test in the clinic the animal models in which we have shown that MIF potentiates the effects of DOPA and alleviates oxotremorine-induced tremors.

TABLE IV. Anticholinergic Study in the Isolated Guinea Pig Ileum with MIF.

Pretreatment MIF (μg/ml)	Av inhibition of acetylcholine HCl (0.04–0.1 μg/ml) (%)
5	10.7
10	8.0
50	4.0
100	35.5

Summary. MIF was demonstrated to antagonize the central and peripheral effects of oxotremorine in normal as well as in hypophysectomized mice. Its effectiveness in hypophysectomized mice confirms our earlier report that MIF exerts actions upon the CNS which are independent of its MSH release-inhibiting activity.

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