STUDIES ON THE INHIBITION OF OXOTREMORINE INDUCED TREMOR BY A MELANOCYTE-STIMULATING HORMONE RELEASE-INHIBITING FACTOR, THYROTROPIN RELEASING HORMONE AND RELATED PEPTIDES

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1. Introduction

In 1967 Cotzias et al. [1] suggested that the melanocyte-stimulating hormone (MSH) of the pituitary may aggravate the tremor in Parkinson's disease and more recently Shuster et al. [2] found increased β-MSH levels in parkinsonian patients. The secretion of MSH from the pituitary is influenced by the hypothalamus [3] and the structure of a hypothalamic melanocyte-stimulating hormone release-inhibiting factor (MIF) has been proposed to be that of a tripeptide, prolylleucylglycinamide (Pro–Leu–Gly–NH_2) [4,5].

Oxotremorine has often been employed to produce in experimental animals a very simple and reproducible model of Parkinson's disease. It has been shown by Everett et al. [6] that L-dopa, used clinically in the treatment of Parkinsonism, will inhibit the tremor induced by oxotremorine. Studies in mice by Plotnikoff et al. [7] have demonstrated that also MIF reduces the tremor caused by oxotremorine and that MIF appears to potentiate the behavioral effects of L-dopa [8].

When evaluated together all these findings seem to indicate that natural MIF may have some influence upon the general syndrome of Parkinson's disease.

In a preliminary clinical study Kastin and Barbeau [9] found that MIF posesses some antiparkinsonian activity, but they concluded that the number of patients studied was not large enough for claiming any therapeutic applications.

The tremor reducing ability of MIF appears to be independent of its MSH-release inhibiting activity, since the tremor was reduced in normal as well as in hypophysectomized mice [7]. In order to investigate whether peptides related to MIF or other hypothalamic neurohormones [3] could antagonize the effects of oxotremorine we have synthesized and tested six tripeptides for inhibition of oxotremorine-induced tremor in mice. These peptides are MIF, the thyrotropin releasing hormone (TRH) of the hypothalamus [10,11], pGlu–Thi–Pro–NH_2 (1) (Thi = β-(2-thienyl)-L-alanine), pGlu–Leu–Gly–NH_2 (*2*) pGlu–Leu–Pro–NH_2 (*3*) and pGlu–Gly–Pro–NH_2 (*4*). (The nomenclature of the amino acids and peptides follow the IUPAC–IUB recommendations 1972).

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2. Materials and methods

The synthesis of the peptides has been effected by classical methods in solution. TRH, which is□—Glu–His–Pro–NH_2, was prepared as described by Chang et al. [12]. □—Glu–Thi–Pro–NH_2 (*1*), □—Glu–Leu–Pro–NH_2 (*3*) and □—Glu–Gly–Pro–NH_2 (*4*) were synthesized as reported by Sievertsson et al. [13]. For these four peptides the reported physical data [12, 13] were again observed.

In the synthesis of MIF and □—Glu–Leu–Gly–NH_2 (*2*) the following steps were utilized. Z–Leu–ONp was coupled with Gly–NH_2 in dimethylformamide (DMF) and the resulting protected dipeptide Z–Leu–Gly–NH_2 was then treated with HBr in HOAc yielding Leu–Gly–NH_2 · HBr. After neutralization with triethylamine the latter dipeptide was coupled with Z–Pro–ONp in DMF, giving Z–Pro–Leu–Gly–NH_2, which after catalytic hydrogenation at room temperature and atmospheric pressure yielded Pro–Leu–Gly–NH_2 (MIF). Leu–Gly–NH_2 was also coupled with □—Glu–OPcp [14] to give □—Glu–Leu–Gly–NH_2 (*2*). Chemical and physical data of these latter peptides are collected in table 1.

Antagonism of oxotremorine induced tremor was estimated using the electronic device described by Silverman and Jenden [15]. The use of this objective method ensures reliable measurements of the tremor intensities [16].

Mice were individually placed in a light bowl resting in the cone of a Quam 16 A6PA moving coil loudspeaker, and an amplifier with a sharp bandpass from 20.0–24.6 Hz was used to amplify selectively the signals generated by the tremor. Signals generated by random movement were rejected. The amplified signal was rectified and integrated over periods of 1 min with an integrator, of essentially infinite time constant, which was reset automatically at 1 min time intervals. The integrator output was recorded on a potentiometric recorder. The total response during a 3 min period following intravenous injection of oxotremorine was used as a measure of the drug response.

The 'up and down' method for small samples described by Dixon [17] was used to estimate the median effective dose of oxotremorine. The response was recorded as positive or negative depending on whether it was greater or less than a predetermined threshold which corresponded approximately to the mean response to 120 μg/kg of oxotremorine.

Oxotremorine was administered to groups of 5 male mice (Swiss-Webstor) weighing 20–29 g and the median effective dose was determined. A logarithmic series of doses of oxotremorine with a spacing of 0.1 units in the Log_{10} dose scale was used. The tested peptides were administered intraperitoneally 1 hr before the oxotremorine, and tremors were measured for a 3 min period, starting 20 sec after the intravenous administration of oxotremorine. Four to five doses including zero were chosen for each peptide.

The median effective dose of oxotremorine was plotted against the dose of the tested peptide (fig. 1). The peptides that showed no significant linear regres-

Table 1
Chemical and physical data of MIF, □—Glu^1–MIF, and intermediary peptides

Peptide	Yield %	R_f^1	R_f^2	R_f^3*	m.p. (°C)/$[\alpha]_D^{20}$
Z–Leu–Gly–NH_2	96	–	0.73	0.82	78°/–9.8 (c 2.5, $CHCL_3$)**
Leu–Gly–NH_2·HBr	100	0.75	0.58	0.19	–
Z–Pro–Leu–Gly–NH_2	37	0.95	0.92	0.85	162–163°/–71.1 (c 1.4,EtOH)***
Pro–Leu–Gly–NH_2(MIF)	89	0.72	0.18	0.68	112–113, 5°/–48.8 (c 1.0,MeOH)†
□—Glu–Leu–Gly–NH_2 (2)	31	0.83	0.85	0.57	175–176°/–17.0 (c 1.0,MeOH)††

* R_f^1, R_f^2 and R_f^3 refer to tlc on silica gel (Merck F 254 precoated plates) in HOAc:EtOAc:BuOH:H_2O (1:1:1:1), EtOH:H_2O:EtOAc (7:4:8) and $CHCl_3$:MeOH:NH_3 (50:20:5) respectively (R_f^1 0.62, R_f^2 0.73, R_f^3 0.23 for Phe).

** Lit. 80°/–10.5 (c 1.8, $CHCl_3$) [21].

*** Lit. 163°/–71.9 (c 2.5, EtOH) [21]; 163° [22,23].

† Lit. 120° [21]; 122–123° [23].

†† Lit. 174–175°/–17.7 (c 0.9, MeOH) [24].

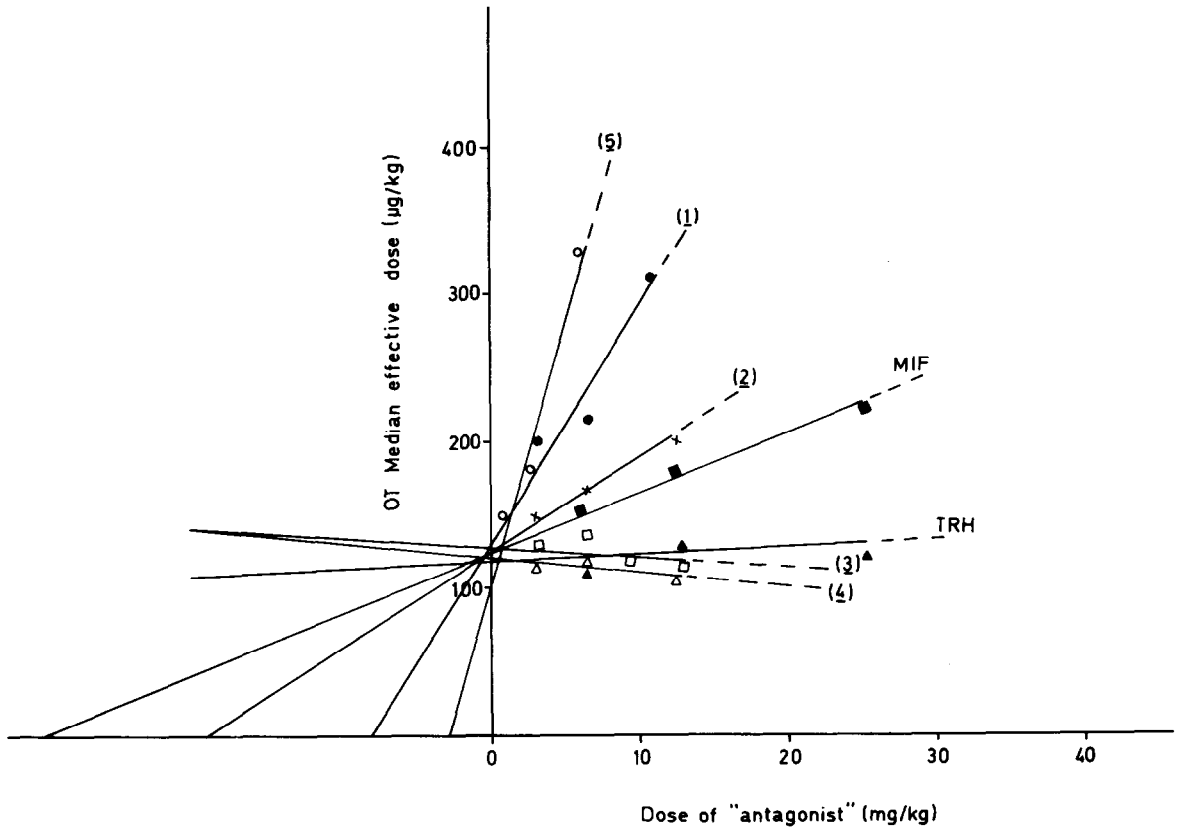
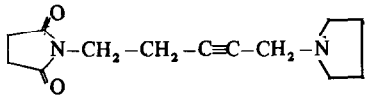


Fig. 1. Median effective dose of oxotremorine (OT) estimated by the 'up and down' method for small samples, plotted against the dose of 'antagonist'. The figures within parentheses refer to the peptides described in the text.

Table 2
Pharmacological results in mice

Compound		In vivo dose (i.p.) to produce oxotremorine blockade	
		mg/kg	µmol/kg
Pro–Leu–Gly–NH_2	MIF	30 (22–42)	105**
pGlu–His–Pro–NH_2	TRH	inactive	
pGlu–Thi–Pro–NH_2	(*1*)	8.0 (3.0–19)	21*
pGlu–Leu–Gly–NH_2	(*2*)	19 (13–31)	65**
pGlu–Leu–Pro–NH_2	(*3*)	inactive	
pGlu–Gly–Pro–NH_2	(*4*)	inactive	
	(*5*)	2.9 (0.3–8.8)	12*

* 90% Confidence limits.
** 95% Confidence limits.

sion at the doses tested are recorded as inactive (table 2).

3. Results and discussion

The data on the inhibition of the oxotremorine induced tremor in mice are summarized in table 2. Fig. 1 shows the assay results after regression analysis. The intercept on the abscissa describes the dosage of the peptide which requires a doubling of the median effective dosage of oxotremorine. Compound *5* has been utilized as a control since this compound is a specific antagonist to oxotremorine [16] and it has also been selected for further evaluation in clinical trials.

As seen in table 2, MIF will inhibit the oxotremorine induced tremor at a dosage of 105 μmol/kg. The replacement of the Pro moiety in MIF with □—Glu gives a peptide □—Glu–Leu–Gly–NH_2 (*2*) which is almost twice as potent as MIF. TRH and its two analogs Leu^2–TRH (*3*) and Gly^2–TRH (*4*) are completely inactive, while Thi^2–TRH (*1*) is about 5 times as potent as MIF. Comparison on a molar basis shows that compound *5* is only about twice as active as Thi^2–TRH (*1*). The high potency of Thi^2–TRH (*1*) in comparison with the inactivity of TRH is interesting since (*1*) is a very active analog of TRH in releasing thyrotropin (TSH) from the pituitary. Hence when tested in mice Thi^2TRH (*1*) has about 30% of the activity of TRH and in an in vitro system, using isolated rat pituitaries, (*1*) shows as much as 77% of the activity of TRH [18]. Leu^2–TRH (*3*) is also a rather potent analog of TRH having about 2% of its activity [18], while Gly^2–TRH (*4*) has no TRH activity [13].

Redding et al. [19] have recently demonstrated that the half-life of MIF in plasma in rats is approximately 9 min and that no intact tripeptide was found in the urine 1 hr after the administration. The first step in the inactivation appears to be the cleavage of the Pro–Leu bond with formation of Pro and Leu–Gly–NH_2. The higher potency of □—Glu–Leu–Gly–NH_2 (*2*) in comparison with MIF may therefore be due to an increased stability against plasma inactivation. Since we administered MIF 1 hr before the administration of oxotremorine it is likely that inhibition of the tremor is due to a secondary process induced by MIF and not by a competitive inhibition as is the case with compound *5* [16]. The inactivity of TRH in this study in contrast to the high activity of its potent analog Thi–TRH (*1*) could indicate that MIF and Thi^2-TRH are acting differently.

One of the effects of L-dopa in the treatment of Parkinson's disease appears to be the replenishing of depleted dopamine in extrapyrimidal centers and thereby increasing the amount of dopamine available to the receptors [20]. The demonstration that MIF, in experimental animals, potentiates the behavioural effects of L-dopa and presumably accelerates the turnover rates of dopamine [8] may indicate a mechanism of action of MIF to inhibit a Parkinson-like tremor. Further evaluation of MIF or other peptides, like those described in this communication, may suggest beneficial effects in the treatment of parkinsonian patients.

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