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On the Optimal Dosage of Pro-Leu-Gly-NH_2 (MIF) in Neuropharmacological Tests and Clinical Use

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Summary. Pro-Leu-Gly-NH_2 (MIF) inhibits the tremor induced by oxotremorine. Objective measurement of this tremor permits the drawing of a dose-effect curve. The inhibitory effect of the peptide increases linearly with increasing doses until an optimum is reached (between 30 and 40 mg/kg i.p.). At still higher doses the peptide is inactive. The same phenomenon is observed with analogues of MIF. This finding may have important bearings on the interpretation of clinical and experimental data obtained with MIF.

Key words: MIF – Melanostatin – Oxotremorine – Parkinson – Peptides.

INTRODUCTION

Prolylleucylglycine amide (MSH Release Inhibiting Hormone, melanostatin, MIF) is a peptide with pronounced direct effects on the central nervous system (Plotnikoff et al., 1971, 1973, 1974a; Huidobro-Toro et al., 1975; Plotnikoff and Kastin, 1976). It is well established (Plotnikoff et al., 1972; Plotnikoff and Kastin, 1974b; Castensson et al., 1974) that MIF reverses the central and peripheral effects of oxotremorine, a centrally acting cholinergic substance which in experimental animals gives rise to a status resembling Parkinson's disease. It has also been shown (Kastin and Barbeau, 1972; Fisher et al., 1974; von Gerstenbrand et al., 1975; Barbeau et al., 1976) that MIF may ameliorate the symptoms of clinical parkinsonism. In view of the potential therapeutic use of MIF we wish to report an important anomaly in the dose-effect relationship of this peptide and of peptides related to MIF, as measured by their ability to inhibit oxotremorine-induced tremor in mice.

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MATERIALS AND METHODS

Antagonism of oxotremorine-induced tremor was measured by means of an electronic transducer (Silverman and Jenden, 1970).

Mice were individually placed in a light bowl resting in the cone of a moving coil loudspeaker, and an amplifier with a sharp bandpass from 19 to 29 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 20 s, after each of which the integrator was reset automatically. The integrator output was recorded on a potentiometric recorder. The tremor was measured for a 3 min period starting 20 s after the i.v. administration of oxotremorine.

The "up and down" method (Dixon, 1965) for small samples was used to calculate the median effective dose of oxotremorine. The response to a single injection was recorded as positive or negative depending on whether it was greater or smaller than a predetermined tremor level approximately corresponding to the mean response to 130 µg/kg of oxotremorine. A logarithmic series of doses with a spacing of 0.1 units in the Log_{10} scale was used.

The tested peptide was dissolved in saline and administered intraperitoneally to male NMRI mice weighing 20–25 g. (Six mice were used at each dose level.) 1 h later the median effective dose of oxotremorine was determined. This was plotted against the dose of the tested peptide and the linear regression lines were calculated up to as high a dose as possible.

The peptides were synthesized and characterized as previously described (Björkman et al., 1976).

RESULTS AND DISCUSSION

Figure 1 shows a typical dose-effect graph of Pro-Leu-Gly-NH_2 (MIF) in our oxotremorine antagonism test. The inhibitory effect of the peptide increases linearly with dose until a maximum of somewhere between 30 and 40 mg/kg is reached. At higher doses the effect then drops to the zero level.

The same phenomenon is observed with the MIF analogues pGlu-Leu-Gly-NH-R (R = H, C_2H_5 or

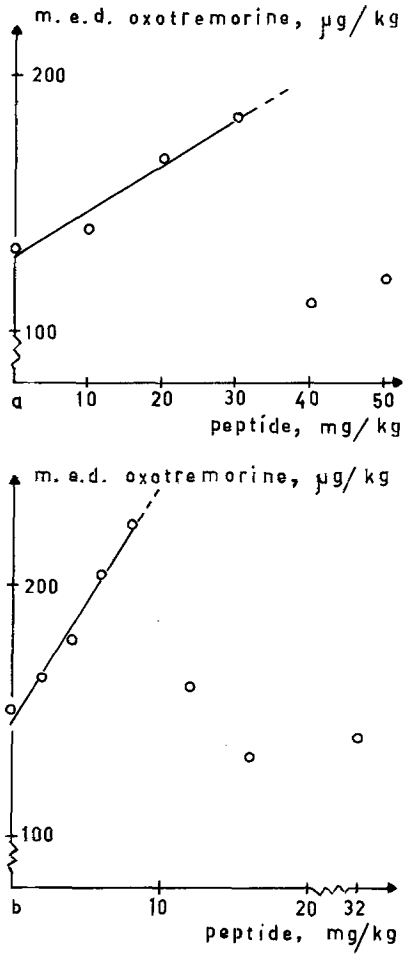

Fig. 1. The dose-effect graphs of Pro-Leu-Gly-NH_2 (a) and pGlu-Leu-Gly-NHC_2H_5 (b). Linear correlation coefficients: in a: 0.98, in b: 0.99

C_3H_7). Figure 1b depicts the dose-effect relationship of pGlu-Leu-Gly-NHC_2H_5 (Björkman et al., 1976). This peptide is 4–5 times more active than MIF and reaches its optimal effect at a correspondingly lower dose (9–11 mg/kg).

These data definitely confirm some trends already reported in the literature but not fully recognized, since in these experiments the results could not be accurately quantified. In a Dopa potentiation test (Plotnikoff et al., 1971) the effect of MIF apparently decreased at a dose of 8 mg/kg or more, which was interpreted as a sedative effect of MIF. In a following study Plotnikoff et al. (1973) found that 1–10 mg/kg of MIF markedly reversed deserpidine-induced sedation in monkeys while 30 mg/kg had only moderate or slight effect. In a double-blind trial on parkinsonian patients a dose of 500 mg/day per os of MIF significantly improved motor performance, while the therapeutic response to 1–1.5 g/day was rather poor (Barbeau et al., 1976).

Attempts to correlate CNS effects of MIF with changes in tissue levels, biosynthesis or degradation of catechol amines have yielded conflicting results. Friedman et al. (1973) reported that MIF (0.5–5 mg/kg i.p.) stimulates dopamine synthesis in corpus striatum (rats). This finding could neither be confirmed by Plotnikoff et al. (1974a), who measured dopamine levels in the whole brain and caudate nucleus, after 100 mg/kg (i.p.) and in whole mouse brains after 10–80 mg/kg i.p. of MIF, nor by Kostrzewa et al. (1975), who employed a peptide dosage of 3 × 20 mg/kg i.p. However, these results might not be conflicting in view of the peculiar dose-effect curve of MIF described in this report.

The identical pattern of the dose-effect curves of MIF and of the more active tripeptide analogs (Fig. 1) suggests that these peptides act by the same mechanism. This adds to our previous finding that a tripeptide amide backbone is important for the tremor inhibiting effect of MIF and its analogues (Björkman et al., 1976).

The mechanism of action of Pro-Leu-Gly-NH_2 on the CNS remains obscure, and consequently no comprehensive explanation can be offered to the phenomenon reported here. However, if the peptide acts as a modulating factor on neural transmission the effects of low drug concentrations on sensitive neuronal receptors might very well be cancelled by the effects of high drug concentrations on other, less sensitive ones. The latter should then be located on neurons whose activities balance those of the neurons bearing the more sensitive receptors.

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