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A comparison of the effects of Pro-Leu-Gly NH_2 and L-leucine on tremorine-induced tremor and rigidity in rats

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The mechanism of action of the potential anti-Parkinson tripeptide Pro-Leu-GlyNH_2 (PLG) remains obscure despite numerous animal studies. Tremorine, and its active metabolite oxotremorine, provide an animal model for testing potential anti-Parkinson agents. Acute administration of PLG was reported to antagonize tremorine-induced tremor in rats (Plotnikoff et al 1972) and oxotremorine-induced tremor in mice (Plotnikoff & Kastin 1974), although others were unable to confirm these findings (Kruse 1977; Björkman et al 1980). A recent study (Dickinson et al 1981) has demonstrated a partial antagonism of tremorine tremor and rigidity in rats treated chronically with PLG, whereas acute doses of the peptide were ineffective. One explanation of these findings may be the accumulation of an active metabolite of PLG during chronic treatment with the peptide. PLG has a short half life in rats and the only significant metabolite is L-leucine (Witter et al 1980). This paper compares the effects of chronic administration of PLG and L-leucine on tremorine tremor and rigidity in rats. Tremorine was used rather than oxotremorine because preliminary studies had established that tremorine produces more sustained tremor and rigidity.

Methods

Female Sprague Dawley rats, 180–220 g, were used. Pro-Leu-GlyNH (PLG, Sigma, 2 mg kg^{-1}) and L-leucine (Sigma, 1 mg kg^{-1}) were dissolved in 0·9% (NaCl saline) solution and administered i.p. once daily for 5 days. Control rats were given saline. Every rat was given 1 mg kg^{-1} of atropine methylnitrate 15 min before tremorine.

Normal limb tone and tremorine-induced rigidity were measured using a mechanical apparatus (Dickinson et al 1981) which measured the force required to partly flex one hind leg of a conscious, lightly restrained rat. Rats were placed singly in the apparatus and 10–12 measurements min^{-1} of limb resistance to flexion were taken for 5–10 min. Tremorine dihydrochloride (20 mg kg^{-1} u.p.) was administered without removing the rat and limb tone measurements made for 35 min.

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Tremor was recorded from one hind leg of rats held singly in a plastic, cylindrical restraining box with 2 slots through which the hind legs hung free. A small permanent magnet was taped to one leg. The leg was surrounded by a coil of 1400 turns of 0·5 mm polyurethane-coated copper wire. Limb movements induced a proportional voltage in the coil which was amplified by a Grass 7P1 preamplifier. The circuit included a filter which eliminated the large voltages induced by gross movements so that only tremors were recorded. The amplified, filtered d.c. signal was divided. One signal was integrated (Grass 7P10) to provide a chart record of tremor intensity measured at 1 min intervals. The other signal triggered a pulse generator. Each tremor generated a constant pulse. The pulses were accumulated by a calibrated (0–25 Hz) 7P10 integrator which re-set every min. A chart record displayed the average tremor frequency.

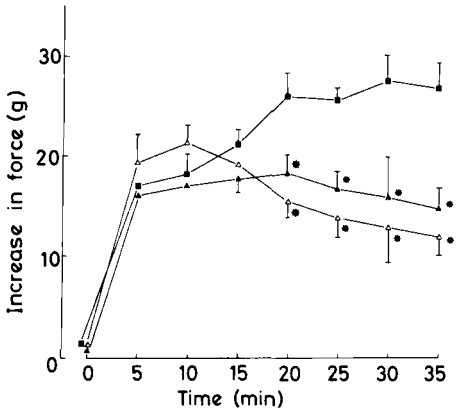

FIG. 1. Effects of PLG and L-leucine on tremorine-induced increase in hind limb muscle tone in rats. Tremorine dihydrochloride (20 mg kg^{-1}) was administered at time 0 to 8 saline-treated rats (■——■), 10 rats pretreated with PLG 2 mg kg^{-1} once daily for 5 days (▲——▲), 10 rats pretreated with L-leucine 1 mg kg^{-1} once daily for 5 days (△——△). Each point is the mean increase in the force required to displace 1 hind leg. Vertical bars show s.e.m. Significance of difference between saline and PLG/leucine groups at the corresponding time interval $^*P < 0·5$ (Student's unpaired, 2-tailed *t*-test).

Table 1. Effects of PLG and leucine on tremorine-induced tremor in rats. PLG (2 mg kg^{-1}) were given daily for 5 days. Tremor was measured 15–20 min after 20 mg kg^{-1} of tremorine dihydrochloride.

Treatment	n	Tremor intensity (arbitrary units min^{-1} ± s.e.m.) Normal	Post-tremorine	Tremor frequency (Hz ± s.e.m.) Normal	Post-tremorine
Saline	18	9·1 ± 0·6	39·6 ± 2·3	3·3 ± 0·5	8·2 ± 0·5
PLG	13	9·2 ± 0·5	27·3 ± 3·1*	3·4 ± 0·1	6·8 ± 0·6
Leucine	11	5·5 ± 0·8	25·5 ± 4·6*	4·9 ± 0·4	7·7 ± 0·5

* $P < 0·05$ compared with saline treated.

The average force required to partly flex one hind leg of saline-treated rats was 47·3 ± 2·9 g (mean of 12 ± s.e.m.). Pretreatment of rats with PLG and L-leucine for 5 days had no effect on the resistance of the hindlimbs to flexion.

Tremorine increased hind leg muscle tone. The time course of the rigidity produced by 20 mg kg^{-1} of tremorine is shown in Fig. 1. Rigidity reached a maximum after 20 min. Pretreatment of rats with PLG partly prevented the rigidity. A closely similar effect was recorded in rats pretreated with L-leucine (Fig. 1).

Preliminary experiments had shown that peak tremor intensity occurred 15–20 min after tremorine administration. The intensity and frequency of spontaneous movements were measured 5 min before tremorine administration. Normal rats had an approximately 3 Hz resting tremor of low intensity (Table 1). Pretreatment with PLG and L-leucine had no significant effects on the spontaneous limb movements.

Tremorine produced a high intensity, 8–9 Hz tremor in the hind limbs of saline-treated rats (Table 1). Pretreatment of rats with PLG for 5 days partly antagonized the peak tremor intensity without affecting significantly the average tremor frequency. L-Leucine pretreatment had a closely similar effect; the tremor intensity was reduced significantly whilst the predominant frequency of tremor was unchanged (Table 1).

Discussion

The findings demonstrate that PLG and its major metabolite L-leucine partly antagonize tremorine-induced rigidity and tremor in rats. Other fragments or potential metabolites of PLG, in particular Leu-Gly-NH_2 and cyclo (Leu-Gly) have been attributed with c.n.s. actions (Van Ree & De Wied 1976; Flexner et al 1977; Walter et al 1978, 1979). Metabolism studies with rat and mouse brain homogenates have shown that PLG is readily cleaved to its constituent amino acids, the major products being leucine and proline (Marks & Walter 1972; Neidle et al 1980). It is unlikely that PLG prevents the hepatic conversion of tremorine to oxotremorine because a previous study has shown that single doses of PLG given intraventricularly entirely prevented tremorine tremor whereas single systemic doses were ineffective (Dickinson & Slater 1981). Furthermore, PLG and some tripeptide analogues are potent antagonists of oxotremorine tremor in mice (Plotnikoff et al 1972; Plotnikoff & Kastin 1974; Björkman et al 1979).

The present findings may partly explain why the central actions of PLG in rats persist up to 24 h after a single dose (Plotnikoff et al 1971) despite the short half life (less than 30 min) of the peptide (Witter et al 1980). Furthermore, the accumulation of metabolites including leucine during chronic PLG treatment may explain why chronic treatment is more effective than acute doses (Dickinson et al 1981; Chiu et al 1981). The metabolites may contribute to the pharmacological actions of PLG.

Although this is the first report that PLG and leucine have similar actions in vivo, it was recently shown that both compounds antagonized 5-HT induced contractions of isolated smooth muscle (Dickinson & Slater 1981). It is intended to extend the present investigation to include other PLG metabolites and also to determine if the in vivo actions of L-leucine are stereospecific.

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