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BRIEF COMMUNICATION

Oxotremorine Antagonism by Prolyl-Leucyl-Glycine-Amide Administered by Different Routes and with Several Anticholinergics

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PLOTNIKOFF, N. P. AND A. J. KASTIN. *Oxotremorine antagonism by prolyl-leucyl-glycine-amide administered by different routes and with several anticholinergics.* PHARMAC. BIOCHEM. BEHAV. 2(3) 417–419, 1974. – Prolyl-leucyl-glycine amide (PLG) was given by oral, intravenous, or subcutaneous routes of administration and shown to antagonize the central and peripheral effects of oxotremorine. The previously demonstrated action of PLG in this system when injected intraperitoneally in a single dose was found to be unchanged when administered by the same route for five consecutive days. Although three anticholinergic compounds were also effective in reducing the effects of oxotremorine, no interactions between PLG and trihexyphenidyl hydrochloride, benzotropin mesylate, or scopolamine were observed. The results confirm and extend the demonstration of the extra-endocrine effects of PLG.

Oxotremorine Parkinson's disease Anticholinergics Hypothalamic peptides Prolyl-leucyl-glycine amide

IN ONE of our original studies of the extra-endocrine effects of prolyl-leucyl-glycine amide (PLG), we reported that the intraperitoneal (i.p.) injection of PLG antagonized the central and peripheral effects of oxotremorine in mice [3]. More recently, Kastin and Barbeau reported that PLG reduced rigidity and tremors in parkinsonian patients [1]. The present investigation represents a further characterization of this activity of PLG on tremors in mice when given by several routes of administration by itself and in combination with other known antiparkinsonian agents.

MATERIALS AND METHOD

Normal (ICR) male mice (16–20 g) were pretreated with varying doses of PLG 1, 4, 8, 24, or 48 hr before administration of oxotremorine (0.5 mg/kg i.p.). The responses of the mice were recorded by blind observational techniques and were compared with those observed in mice which received only oxotremorine as a control [3]. The responses were graded as 0 (no effect) 1 (slight), 2 (moderate), and 3 (marked) for the effect of oxotremorine on each group of mice (4 mice per group). The scores for the central effects of oxotremorine (including increased motor activity, tremors, head twitch, limb abduction, and ataxia) were added together with the scores of the peripheral effects (lachrymation, salivation, and diarrhea). The antagonism of oxotremorine by PLG was estimated by a reduction in these total scores as compared with controls receiving only the oxotremorine.

RESULTS

Oral Route

Substantial reduction of the effects of oxotremorine 1, 4, and 24 hr after its administration was observed when PLG was administered by oral intubation (Table 1).

Intravenous Route

PLG, given intravenously (i.v.) one hr earlier effectively antagonized the effects of oxotremorine at a dose of 10 mg/kg (score: 11), 20 mg/kg (score: 9), and 40 mg/kg (score: 7) as compared with controls (score: 22).

TABLE 1

ANTAGONISM OF OXOTREMORINE BY PLG ADMINISTERED BY THE ORAL ROUTE

Dose PLG (mg/kg)	Total Score 1 hr	4 hr	24 hr	48 hr
0	21	21	22	22
0.5	21	21	–	–
1	17	18	–	–
2	15	16	–	–
2.5	–	–	22	–
4	12	13	–	–
5	–	–	22	22
8	10	11	–	–
10	–	–	8	22
16	8	8	–	–
20	7	7	16	19
40	6	7	9	18

TABLE 2

ANTAGONISM OF OXOTREMORINE BY PLG AND TRIHEXYPHENIDYL OR PLG AND BENZTROPIN

		Dose of PLG (mg/kg i.p.) 0	5	10	20
Dose trihexyphenidyl (mg/kg i.p.)	0	20	16	16	10
	5	11	7	8	10
	10	8	5	7	4
	20	6	1	0	1
Dose benztropin (mg/kg i.p.)	0	20	16	16	10
	1.25	6	6	6	6
	2.5	4	1	3	1
	5.0	0	1	2	0

TABLE 3

ANTAGONISM OF OXOTREMORINE BY PLG, SCOPOLAMINE, AND L-DOPA

Treatment	Dose (mg/kg i.p.) Scopolamine	PLG	L-DOPA	Score
	0	0	0	20
Scopolamine only	0.5	0	0	17
	1.0	0	0	13
	2.0	0	0	6
Scopolamine and DOPA	0.5	0	100	10
	1.0	0	100	8
	2.0	0	100	5
PLG only	0	5	0	20
	0	10	0	10
	0	20	0	7
PLG and DOPA	0	5	100	14
	0	10	100	8
	0	20	100	5
PLG, DOPA and Scopolamine	0.5	5	100	8
	0.5	10	100	5
	0.5	20	100	4

Subcutaneous Route

PLG was also found to be effective in antagonizing the effects of oxotremorine one hour later when given by the subcutaneous route in a dose range of 10–40 mg/kg which resulted in scores of 10–13.

Intraperitoneal Route

PLG, as expected, was effective in reducing the responses induced by oxotremorine when administered by the i.p. routes 8 and 24 hr earlier. Doses of 10–40 mg/kg reduced the scores from 22 to 9–12. This action was only slightly improved by administration of PLG for 5 consecutive days (score: 8–9).

Interactions with Anticholinergic Drugs

The effects of trihexyphenidyl hydrochloride (Artane) and PLG alone and in combination were tested. Scores resulting from the different mixtures of the two compounds are shown in Table 2. An analysis of variance followed by

Duncan's multiple range test shows a significant dose effect of even the smallest dose (5 mg/kg) of trihexyphenidyl as well as one of PLG, but no significant interaction.

A similar analysis was also done on the effects of benztropin mesylate (Cogentin) with PLG. Table 2 shows the results. Benztropin, like trihexyphenidyl, was found to have a significant dose-response effect. PLG showed no significant combined effect with benztropin.

Analysis of variance was also used to evaluate the effects of scopolamine, PLG, and L-DOPA. The doses and scores are given in Table 3. Scopolamine and PLG each showed significant ($p<0.01$) actions in antagonizing the effects of oxotremorine. The interaction effect of administration of PLG together with L-DOPA tended ($p = 0.10$) to be greater than when either compound was injected by itself.

DISCUSSION

The present study demonstrates that PLG is effective in antagonizing the effects of oxotremorine when administered by the oral, intraperitoneal, intravenous, or subcutaneous routes. Of unusual interest was a duration of action greater than 24 hours when PLG was administered by either the oral or intraperitoneal route. The half-life disappearance of PLG in rats [5] and man [4] has been shown to be less than 10 minutes. In view of these findings, repeated dosing of animals for five days was undertaken to determine whether there were any accumulative effects of PLG. No evidence was obtained after 5 days medication of either accumulative effects or tolerance. There appears to be a minimum dose for substantial activity (10 mg/kg) by all routes tested, which might suggest specific receptor binding. Since our earlier studies, [2,3] indicated marked potentiation of DOPA, it is possible that PLG is acting on dopaminergic receptors. Further indirect support for this hypothesis is the finding reported here that PLG did not increase the actions of the anticholinergic drugs – trihexyphenidyl, benztropin, and scopolamine – in antagonizing the effects of oxotremorine.

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