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Neuroleptic Drug–Induced Dopamine Receptor Supersensitivity: Antagonism by L-Prolyl-L-Leucyl-Glycinamide

Abstract. *An animal model of tardive dyskinesia was used to evaluate the potential antidyskinetic properties of the neuropeptide L-prolyl-L-leucyl-glycinamide (PLG). In rats, PLG administered concurrently with the neuroleptic drug haloperidol or chlorpromazine antagonized the enhancement of specific [^{3}H]spiroperidol binding in the striatum that is associated with long-term neuroleptic treatment. The results are discussed in relation to a possible functional coupling of the putative PLG receptor with neuroleptic–dopamine receptor complex and clinical implications for tardive dyskinesia.*

Antipsychotics belonging to the chemical classes of butyrophenone, phenothiazine, and thioxanthene are thought to exert their therapeutic effects by selectively blocking central dopamine receptors (*1*). Prolonged neuroleptic therapy in the management of psychiatric patients, however, has produced a variety of extrapyramidal motor disorders, tardive dyskinesia being the most prevalent. Animal models of tardive dyskinesia have been developed in rodents and nonhuman primates, and such studies suggest that supersensitivity of dopaminergic neuronal systems in the basal ganglia is the primary mechanism of tardive dyskinesia (*2*). Diverse pharmacological approaches have been attempted to reverse or prevent tardive dyskinesia, but none has yielded a consistent and favorable clinical outcome (*3*); differential modification of the sensitivity of dopamine receptors remains the preferred theoretical basis for designing therapeutic agents to alleviate tardive dyskinesia.

Clinical studies indicated that lithium and L-prolyl-L-leucyl-glycinamide (PLG) transiently but significantly reduced the intensity of dyskinetic symptoms associated with protracted antipsychotic therapy (*4*). In rats, prolonged lithium treatment in conjunction with neuroleptics abolished both the biochemical and behavioral manifestations of dopaminergic supersensitivity: such treatment enhanced neuroleptic–dopamine receptor binding and augmented stereotyped behavioral responses toward dopamine agonists (*5*). The potential antidyskinetic properties of PLG, a neuropeptide derived from the carboxyl terminal of oxytocin (*6*), have not been critically investigated. We found that PLG antagonized morphine- (*7*) and haloperidol-induced (*8*) catalepsy and selectively enhanced the affinity of the [^{3}H]apomorphine binding to neuroleptic-dopamine receptors in rat striatum (*8*). To investigate the possible desensitizing effect of PLG on dopaminergic supersensitivity, we studied the chronic effects of PLG, when administered concurrently with haloperidol or chlorpromazine, on dopamine receptor function, as measured by [^{3}H]spiroperidol binding in rat striatum.

Two series of drug studies were undertaken in male Sprague-Dawley rats to examine (i) PLG-haloperidol interaction and (ii) PLG-chlorpromazine interaction. The experimental protocols for the drug treatments of various groups of animals are described in Tables 1 and 2.

The procedure of Creese *et al.* (*9*) was adopted for the *l*-[phenyl-4-^{3}H]spiroperidol (25.64 Ci/mmole; New England Nuclear) binding assay. The specific binding of [^{3}H]spiroperidol was defined as the difference in binding in the presence and absence of 500 n*M* of unlabeled spiroperidol. The binding data were analyzed by the Scatchard plot from which the binding parameters, maximal number of binding sites (B_{max}), and dissociation constant (K_d) were derived by linear regression analysis. The biochemical data from different groups of animals were analyzed statistically by one-way analysis of variance followed by the Duncan multiple range test.

Our results indicate that Scatchard plots obtained from normal saline-control rats yielded a single class of noninteracting binding sites with a B_{max} of 317 ± 25 fmole per milligram of protein and K_d of 0.52 ± 0.20 n*M*. Protracted treatment with haloperidol and chlorpromazine resulted in significant ($P < .05$) elevation of the receptor density of [^{3}H]spiroperidol in rat striatum (Table 1), compared to that in the saline controls. Haloperidol (3 mg/kg, intraperitoneally) administered once daily for 21 days caused a mean increase of 58 percent in the B_{max} of [^{3}H]spiroperidol binding over the saline controls, whereas chlorpromazine (20 mg/kg, intraperitone-

Table 1. Blockade of haloperidol (HAL)-induced increase in specific [^{3}H]spiroperidol binding by L-prolyl-L-leucyl-glycinamide (PLG). Male Sprague-Dawley rats weighing 200 to 250 g were randomly assigned to six groups and received various drug dosages for 21 days according to the following protocol: group 1 was given isotonic saline (1 ml/kg, subcutaneously); groups 2 and 3 were administered PLG at the respective doses of 10 and 40 mg/kg, subcutaneously; groups 4 and 5 were dosed respectively with PLG at 10 and 40 mg/kg, subcutaneously, 10 seconds before administration of haloperidol (3 mg/kg, intraperitoneally); and group 6 received haloperidol (3 mg/kg, intraperitoneally) only. The animals were killed 5 days after the last drug session and [^{3}H]spiroperidol binding was carried out on striata. The striatum from each rat in the different treatment groups was used for one Scatchard plot of [^{3}H]spiroperidol binding from which the mean values and standard errors of B_{max} and K_d were determined.

Group	Treatment	*N*	B_{max} (fmole per milligram of protein)	K_d* (n*M*)
1	Saline	8	317 ± 25	0.52 ± 0.20
2	PLG	8	296 ± 44	0.50 ± 0.11
3	PLG	4	267 ± 29	0.38 ± 0.07
4	PLG and HAL	4	380 ± 14	0.66 ± 0.18
5	PLG and HAL	4	384 ± 20	0.74 ± 0.25
6	HAL	5	498 ± 22†	0.58 ± 0.20

*No statistically significant difference was found among the six treatment groups with respect to the K_d values at .05 level. †Significantly different ($P < .05$) from treatment groups 1, 2, 3, 4, and 5 by Duncan's multiple range test.

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Table 2. Blockade of chlorpromazine (CPZ)-induced increase in specific [^{3}H]spiroperidol binding by PLG. Four randomly assigned experimental groups of male Sprague-Dawley rats were subject to the following schedules of drug treatments once daily for 21 days: group 1 received saline only; group 2 was injected with PLG at 10 mg/kg, subcutaneously; group 3 was administered PLG (10 mg/kg, subcutaneously) 10 seconds before CPZ (20 mg/kg, intraperitoneally); and group 4 was dosed with CPZ (20 mg/kg, intraperitoneally). The animals were killed 5 days after the last drug session and striata were assayed for [^{3}H]spiroperidol binding.

Group	Treatment	N	B_{max} (fmole per milligram of protein)	K_d* (nM)
1	Saline	8	317 ± 25	0.52 ± 0.20
2	PLG	8	296 ± 44	0.50 ± 0.11
3	PLG and CPZ	4	269 ± 33	0.19 ± 0.07
4	CPZ	4	529 ± 41†	0.34 ± 0.20

*No statistically significant difference was found among the four treatment groups with respect to the K_d values at .05 level. †Significantly different ($P < .05$) from treatment groups 1, 2, and 3 by Duncan's multiple range test.

ally) produced a mean increase of 67 percent. These observations confirm the results of other studies (*10*, *11*) on the enhancement of specific binding of ^{3}H-labeled neuroleptics after prolonged administration of the drugs.

Simultaneous administration of PLG with haloperidol or chlorpromazine, however, antagonized the elevation in specific [^{3}H]spiroperidol binding produced by long-term administration of neuroleptics alone. Striata from rats treated with both haloperidol and PLG (10 mg/kg, subcutaneously) exhibited a receptor density for specific [^{3}H]spiroperidol binding that was not significantly different from that found in saline controls or PLG controls (Table 1). Rats concurrently treated with both PLG (10 mg/kg) and chlorpromazine (20 mg/kg) failed to demonstrate the increase in specific [^{3}H]spiroperidol binding associated with treatment with chlorpromazine alone (Table 2). Administration of PLG alone at the dose of 40 mg/kg for 3 weeks produced a slight decrease in specific [^{3}H]spiroperidol binding (Table 1); the difference, however, was not statistically significant (at .05 level) compared with that for the saline-treated groups.

In all the drug treatment groups, no statistically significant difference was found in the K_d of [^{3}H]spiroperidol binding sites in the striatum. Hence the alterations in the sensitivity of neuroleptic-dopamine receptors in the striatum, caused by prolonged neuroleptic administration, and reversal of the changes by cotreatment with PLG are reflected primarily in the relative density of [^{3}H]-spiroperidol binding sites rather than in the affinity of the binding ligand.

The results demonstrate that PLG, when administered concurrently with a prototypal dopamine receptor antagonist (chlorpromazine or haloperidol), effectively antagonizes the development of dopamine receptor supersensitivity by restoring the specific binding of [^{3}H]-spiroperidol toward a normal level in the striatum. Pert *et al.* (*5*) showed that lithium cotreatment suppressed the development of neuroleptic-induced dopaminergic supersensitivity and suggested that the therapeutic action of lithium in manic depression may be related to its ability to stabilize the oscillations of dopamine receptor sensitivity. Both the behavioral and biochemical manifestations of neuroleptic-induced dopaminergic supersensitivity have been found to be reversed (*12*) or antagonized (*13*) by administering dopamine agonists like bromocriptine. In view of the multiple complex interactions of dopamine with other putative neurotransmitters in the basal ganglia, the ability of an agent to exhibit direct agonist activity at the dopamine receptor site is not a prerequisite for the desensitizing process.

It is conceivable that there is a pharmacologically distinct receptor for PLG that is functionally coupled to the neuroleptic–dopamine receptor–adenylate cyclase complex and that activation of the putative PLG receptor is responsible for the observed desensitizing effects of PLG on dopamine receptors. We have already shown (*8*) that PLG selectively increases the affinity of the specific binding of the dopamine agonist [^{3}H]apomorphine to the neuroleptic-dopamine receptor in vitro without affecting specific [^{3}H]spiroperidol binding. We also identified, by a radioligand-binding technique, high-affinity PLG binding sites that exhibited saturability, reversibility, and pharmacological and regional specificity in rat and human brain (*14*). It is relevant to note that *cyclo*(Leu-Gly), a diketopiperazine derivative of PLG, competed for specific PLG binding as actively as it does in vivo, whereas inactive mono- and dipeptides like Leu-Gly and proline failed to affect PLG binding. Bhargava and Ritzmann (*15*) found that *cyclo*(Leu-Gly), when administered before haloperidol, suppressed the behavioral manifestations of haloperidol-induced supersensitivity of dopamine receptors: it augmented locomotor hyperactivity and hypothermic responses toward apomorphine. Although no attempt was made in our study to correlate the temporal profile of biochemical changes with behavioral events, it is likely that the pharmacological activity of both PLG and *cyclo*-(Leu-Gly) is produced through interaction with the putative PLG receptor.

The distribution of endogenous PLG in mammalian brain is of interest in relation to the possible association of the putative PLG receptor with the neuroleptic–dopamine receptor–adenylate cyclase complex. Studies by Kastin *et al.* (*16*) indicate that the pineal constituted one site for the extrahypothalamic distribution of PLG, but detailed topographic mapping of PLG in the brain has not been completed.

Our studies demonstrate the antagonism of neuroleptic-induced dopamine receptor supersensitivity by the neuropeptide PLG. Hence PLG and its derivatives should be considered as potential prophylactic and therapeutic agents in the clinical management of tardive dyskinesia.

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