Pharmacology Biochemistry & Behavior, Vol. 9, pp. 375–378. Printed in the U.S.A.

Potentiation of Apomorphine Action in Rats by L-Prolyl-L-Leucyl-Glycine Amide

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(Received 10 July 1978)

KOSTRZEWA, R. M., A. J. KASTIN AND S. K. SOBRIAN. *Potentiation of apomorphine action in rats by l-prolyl-l-leucyl-glycine amide.* PHARMAC. BIOCHEM. BEHAV. 9(3) 375–378, 1978.—Although the antiparkinsonian activity of l-prolyl-l-leucyl-glycine amide (PLG=MIF-I) has been previously observed in several clinical trials, little is known of the mechanism of action of this tripeptide on the brain. Our study demonstrates potentiation of the action of apomorphine by PLG on the rotational behavior of mature rats which received unilateral 6-OHDA (16 μg) lesions of the striatum as neonates. No change in tyrosine hydroxylase or dopa decarboxylase activities in rat striatal homogenates was found after addition of PLG (10^{-6}–10^{-3} M). The results suggest that PLG modifies the dopamine receptor, making it more responsive to stimulation by the agonistic agent apomorphine and perhaps by the natural neurotransmitter dopamine.

Hypothalamus Prolyl-leucyl-glycine amide MIF-I Peptide Tyrosine hydroxylase Apomorphine Rotation Dopa decarboxylase Amphetamine

THE effectiveness of the hypothalamic tripeptide l-prolyl-l-leucyl-glycine amide (PLG=MIF-I) as an antiparkinsonian agent was indicated several years ago in clinical trials in man [13]. This observation with PLG has since been confirmed by other groups [1, 4, 8, 10], and it has been reported that PLG "far surpassed the clinical effect of any of the numerous antiparkinson drugs . . . tested . . . over the past 15 years, including levodopa alone" [1].

Although PLG appears to be capable of alleviating the symptoms of Parkinson's disease [1, 2, 4, 8, 10, 13], there is conflicting evidence concerning actions of the peptide on dopaminergic neurons. The activity of tyrosine hydroxylase, the rate-limiting enzyme involved in the synthesis of dopamine, reportedly was enhanced in vitro in slices of striatum incubated in the presence of PLG [9]. However, given in vivo, PLG was not found to alter tyrosine hydroxylase activity in either the substantia nigra or striatum of rats [17]. Additionally, uptake of ^{3}H-dopamine in striatal homogenates of rats was unaltered after treatment with PLG [16,17]. Previous studies indicated that the levels of homovanillic acid were unchanged in the striatum of rats receiving PLG [21] and that dopamine turnover was not accelerated by PLG [16]. Two recent investigations would seem to contradict most of these reports by finding that PLG increased dopamine turnover in rat striatal tissue [28] and whole brain [22].

When administered to rats pretreated with pargyline and L-DOPA, PLG markedly enhanced behavioral arousal [11, 12, 20, 21, 25, 29] and simultaneously elevated dopamine to a level two-fold greater than in the group that received only pargyline-L-DOPA [25]. However, PLG apparently did not accelerate this dopamine synthesis through direct activation of the dopa decarboxylase enzyme, since activity of the enzyme was unaltered in the substantia nigra and striatum of PLG-treated animals [17].

These studies, therefore, indicate that the actions of PLG on dopaminergic neurons are uncertain. Although the suggestion has been made that PLG acts at a postsynaptic site of dopaminergic neurons [1–3, 14, 16], there has been a failure of the agent to alter levels of the so-called second messenger cyclic adenosine monophosphate (cAMP) in the striatum [5]. Under some conditions PLG has been found to potentiate the action of apomorphine [2,19], but other studies have not found an interaction [6, 16, 19, 24].

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The present study was undertaken in order to determine whether PLG would modify behavior in an animal model where supersensitivity to dopamine develops in the striatum after lesion of the dopaminergic nerve terminals by the neurotoxin 6-hydroxydopamine [27]. After these unilateral chemical lesions of the striatum, rats exhibit rotational behavior. This behavior results from an imbalance in dopamine receptor activity between the innervated and denervated side, with animals turning away from the side of greater neuronal activity. In unilaterally lesioned rats, drugs like amphetamine which act at presynaptic dopamine terminals induce ipsilateral turning toward the lesioned side. Dopamine receptor agonists such as apomorphine elicit contralateral turning away from the side of the lesion which is believed to reflect the development of postsynaptic denervation supersensitivity on the lesioned side [15, 26, 27, 30]. Our findings demonstrate that PLG potentiates the action of apomorphine. This suggests that PLG can act at a postsynaptic site to enhance the actions of neurally released dopamine.

METHOD

In the behavioral study, Sprague-Dawley rat pups of both sexes received unilateral striatal injections of 6-hydroxydopamine hydrobromide (6-OHDA) (Regis Chemical Co., Chicago, IL) either 1 day or 5 days after birth. The pups were anesthetized with ether and secured in a plasticine holder that was mounted on a stereotaxic instrument. An injection of 16 μg of 6-OHDA in 2 μl of vehicle solution (0.85% NaCl+0.1% ascorbic acid) was delivered over a 2 min period through a 30 gauge needle, displaced 2.0 mm anterior to the bregma, 2.0 mm laterally along the coronal suture, and lowered to a depth of 3.5 mm. Controls received 2 μl of vehicle.

At 90–120 days of age, drug-induced rotational behavior was measured in unilaterally lesioned and control rats 30 min after injection of the following materials: (a) PLG (1 mg/kg IP); (b) d-amphetamine sulfate (4 mg/kg IP); (c) apomorphine hydrochloride (1 mg/kg IP); (d) PLG 30 min before amphetamine; (e) PLG 30 min before apomorphine; and (f) vehicle [0.85% NaCl slightly acidified with acetic acid (0.01 M)]. Each rat was tested under all drug conditions; administration of the drugs was counterbalanced and separated by 5–7 days.

Rotational behavior was tested in a rotometer which consisted of a plastic half sphere. A light harness fitted around the animal's waist was attached to an electromagnetic counter which recorded the number of 360° turns in either direction. Rotational scores were calculated by substracting the number of contralateral turns (away from the lesioned side) from the number of ipsilateral turns (toward the lesioned side) completed during a 30 min test session.

Rotational data were analyzed using a split-plot factorial analysis of variance with one between and one within variable. Post hoc comparisons were made with the Neuman-Keuls test.

In a separate study, the in vitro activities of tyrosine hydroxylase and dopa decarboxylase were determined in rat striatal homogenates in the presence or absence of PLG (10^{-8} to 10^{-3} M). Tyrosine hydroxylase activity was measured by a modified method of Coyle [7,23]. Dopa decarboxylase activity was determined by the method of Lamprecht and Coyle [18]. Dunnet's t test was used to compare treated with control groups.

RESULTS

Both d-amphetamine and apomorphine induced significant turning behavior in adult rats which had received unilateral striatal lesions as neonates, $F(5,50)=19.17$, $p<0.01$. The age at which the rats were lesioned (postnatal Day 1 or Day 5) was not a significant parameter, $F(1,10)=1.46$. Paradoxically, rotations were contralateral for both drugs. However, the increase in the net number of contralateral turns per test session was significantly greater after apomorphine ($p<0.05$). PLG alone did not induce rotational behavior, but pretreatment of rats with PLG resulted in a potentiation of apomorphine-induced turning. Rotational behavior after d-amphetamine was not significantly altered by pretreatment with PLG (Fig. 1).

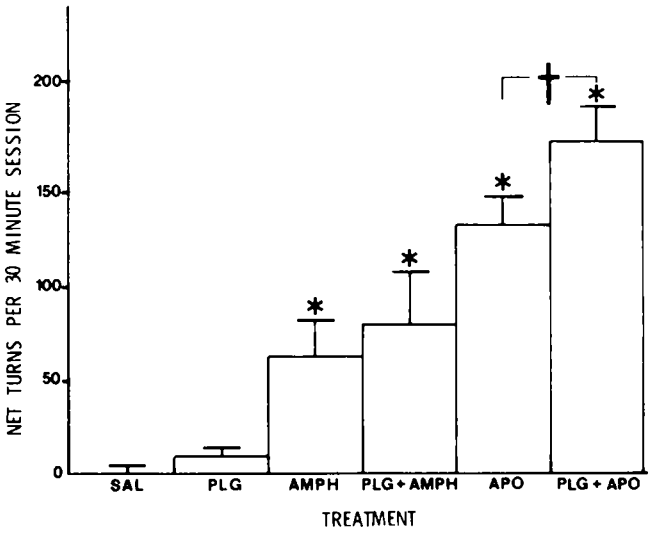

FIG. 1. PLG potentiation of the effect of apomorphine on rotational motor activity of rats with unilateral lesions of the striatum. Rats were lesioned at birth with 6-hydroxydopamine hydrobromide (16 μg/2 μl, free base) and received the following agents at 90 to 120 days of age: saline-acetic acid (0.01 M)=SAL; L-prolyl-L-leucyl glycine amide (1 mg/kg IP=PLG); amphetamine (4 mg/kg IP=AMPH); the combination of PLG and amphetamine=PLG+AMPH; apomorphine (1 mg/kg IP=APO); and the combination of PLG and apomorphine=PLG+APO. Each bar is the mean number of rotations per 30 minute session ± SEM for 7 animals. An asterisk indicates a significant difference from the diluent control, $p<0.01$; a dagger represents a significant difference between indicated groups, $p<0.05$.

The tyrosine hydroxylase activity of rat striatal homogenates remained unchanged after in vitro incubation of the samples with various amounts of PLG (10^{-8} to 10^{-3} M) (Table 1). A similar lack of effect of PLG on dopa decarboxylase activity was also found, as is shown in Table 1.

DISCUSSION

The results of the in vitro part of this report support our earlier in vivo findings that PLG does not alter the activities of tyrosine hydroxylase or dopa decarboxylase in rat striatum. This, as stated previously [14], is at variance with an earlier study by another group [9]. Much of the evidence cited in the introduction indicates that PLG does not directly alter dopaminergic neuronal function, but the issue is confused by varying experimental conditions.

TABLE 1

ACTIVITIES OF TYROSINE HYDROXYLASE AND DOPA DECARBOXYLASE IN RAT STRIATAL HOMOGENATES INCUBATED IN THE PRESENCE OF L-PROLYL-L-LEUCYL-GLYCINE AMIDE (PLG)

PLG Concentration (μM)	Tyrosine Hydroxylase Activity (μM/g tissue/hr)*	Dopa Decarboxylase Activity (μM/g tissue/hr)*
0.00	328.4 ± 26.3	703.2 ± 29.5
0.01	351.4 ± 63.3	724.3 ± 67.4
0.10	331.1 ± 24.8	731.3 ± 92.9
1	354.7 ± 62.1	715.5 ± 59.4
10	315.3 ± 27.9	734.8 ± 97.8
100	333.4 ± 45.0	683.5 ± 22.5
1000	338.3 ± 47.4	646.9 ± 41.5

*Each sample is the mean ± SE of 6 determinations.

In the in vivo part of the present study, PLG was found to potentiate the actions of apomorphine in a motor task associated with the supersensitivity of dopamine receptors. This supports the hypothesis that PLG may be effective in Parkinson's disease because of an action at the dopaminergic receptor. Preliminary evidence of similar hypermotility after apomorphine and PLG has been mentioned [2,3].

There is other indirect evidence that PLG does not release dopamine from the nerve terminals [6,19] or alter dopamine turnover in the striatum [16]. Moreover, our behavioral data show that PLG did not potentiate amphetamine-induced rotational behavior. The contralateral turning that we observed after administration of amphetamine is in contrast to previous reports that amphetamine induces ipsilateral rotation in rodents with chemical lesions of the striatum [15, 26, 27, 30]. One possible explanation is that in neonatally lesioned rats some regenerative sprouting occurs after 6-OHDA resulting in a supersensitive striatum with some presynaptic input.

The suggestion of an action of PLG at the dopaminergic receptor is consistent with the striking findings with PLG in the dopa-potentiation task [11, 12, 20, 21, 25, 29]. In this test, PLG could associate with the dopaminergic receptor and modify it in such a manner as to enhance the actions of dopamine (as with apomorphine in the present study) and produce a heightened arousal state. At the same time, dopamine levels would tend to increase, as was found [25]. This would be explained by a decreased rate of release of the dopamine transmitter which would occur after the enhanced receptor activation and greater feedback inhibition of dopaminergic neurons.

These experiments, therefore, suggest the possibility that the dopaminergic receptor is altered in rats treated with PLG so that the receptor has a higher affinity for dopamine or is activated to a greater degree by like amounts of dopamine (or dopaminergic receptor agonists). Whether these effects of PLG occur directly, by release of another substance, or indirectly by a metabolite is not known.

ACKNOWLEDGEMENTS

This work was supported by funds from the American Parkinson Disease Association and by the Medical Research Service of the Veterans Administration. We thank Mrs. Julie Lore for typing the manuscript.

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