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Potentiation of Apomorphine-Induced Stereotypies by Naloxone and L-prolyl-L-leucyl-glycinamide

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QUOCK, R. M., T. S. LUCAS AND T. J. HARTL. *Potentiation of apomorphine-induced stereotypies by naloxone and L-prolyl-L-leucyl-glycinamide.* PHARMACOL BIOCHEM BEHAV 19(1) 49–52, 1983.—We compared the influences of pretreatment with the narcotic antagonist drug naloxone and the neuropeptide L-prolyl-L-leucyl-glycinamide (PLG) upon apomorphine-induced stereotypic climbing activity in mice and apomorphine-induced contralateral rotational behavior in rats with unilateral 6-hydroxydopamine lesions of the substantia nigra. Naloxone produced dose-related potentiation in the mouse climbing model, while PLG was without effect. On the other hand, PLG produced dose-related potentiation in the rat rotational paradigm, while naloxone was without appreciable influence. These findings show an asymmetrical potentiation of apomorphine by naloxone and PLG in these two standard experimental models of striatal dopaminergic activity.

Apomorphine Naloxone L-prolyl-L-leucyl-glycinamide Potentiation of dopaminergic drug effects

FOR the past several years, we have been investigating the potentiating influence of narcotic antagonist drugs upon dopaminergic drug effects. Pretreatment with naloxone has been found to enhance apomorphine-induced hyperthermia in rabbits [24], apomorphine-induced turning in rats with unilateral electrocoagulation lesions of the substantia nigra [26], apomorphine-induced stereotypic climbing in mice [25], the anticataleptic effect of l-dopa in reserpinized mice [18] and the antitremor effect of l-dopa in oxotremorine-treated mice [14].

L-Prolyl-L-leucyl-glycinamide (PLG) is a hypothalamic tripeptide which has been shown to participate in regulation of the release of melanocyte-stimulating hormone (MSH) from the pituitary gland [17], hence the synonym melanocyte-stimulating hormone release inhibitory factor (MIF). Further research has demonstrated that PLG is capable of exerting significant neuropharmacological effects independent of its neuroendocrine function. Pretreatment with PLG has been found to potentiate apomorphine-induced contralateral rotations in rats with unilateral 6-hydroxydopamine lesions of the corpus striatum [13], apomorphine-induced mounting behavior in rats [19], l-dopa suppression of harmine-induced tremors in rabbits [12] and the effects of l-dopa on motor performance in human parkinsonian subjects [2]. In addition, it has now been reported that this same neuropeptide apparently exerts narcotic antagonist properties as it can reverse morphine-induced analgesia [7] and catalepsy in rats [3].

Since both naloxone and PLG appeared to share dopaminergic potentiatory and narcotic antagonistic properties, we decided to compare their influences and potencies in two experimental models of central dopaminergic activity. Herein we will report an asymmetrical potentiation by naloxone and PLG of apomorphine-induced stereotypic climbing activity in mice and apomorphine-induced rotational behavior in rats with unilateral lesions of the substantia nigra.

METHOD

Experiments in Mice

Two hundred male ICR mice (Harlan Laboratories, Indianapolis, IN), 18–25 g, were used in these experiments. They were individually acclimated for 30 min to specially built circular cages (12 cm in diameter and 14 cm in height, ringed by 1-mm metal bars spaced 1 cm apart). These cages were separated by cardboard screens to prevent behavioral interaction between animals. Following administration of a standard challenge dose of 2.0 mg/kg of apomorphine, the mice were watched for a 20-min observation period. Assessment of apomorphine-induced stereotypic climbing was made according to a previously reported method [23]: 0 points, the animal sits with all four paws on the cage floor; 1 point, the animal persistently stands against the cage wall with forepaws grasping the bars; and 2 points, the animal persistently climbs on the cage wall with all four paws grasping the bars. Climbing scores were taken during 5-min periods ending 10 and 20 min after the apomorphine challenge and the two scores were then averaged.

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Experiments in Rats

Eight male Sprague Dawley rats (Harlan Laboratories, Indianapolis, IN), 200–300 g, were used in these experiments. During sodium pentobarbital anesthesia, each rat was mounted in a stereotaxic headholder (David Kopf Instruments, Tujunga, CA) and 8 μg of 6-hydroxydopamine hydrobromide (6-OH-DA as the base) (Regis Chemical Company, Morton Grove, IL) in 1.5 μl of 0.1% ascorbic acid were microinjected over a 2-min period into the substantia nigra unilaterally at stereotaxic coordinates 2.9 mm A, −2.5 mm V and 1.7 mm L [28]. Rotational behavior experiments were begun 3 weeks following surgery. Each rat was subjected to a randomized sequence of five experiments (one control, two doses of naloxone and two doses of PLG) with 4-day intervals between experiments. The entire sequence of experiments was performed at least 3 times in each animal. The rat was placed into a hemispherical Plexiglas bowl and acclimated for at least 30 min. Following administration of a standard challenge dose of 1.0 mg/kg of apomorphine, the animal was observed for 30 min. The number of rotations for fifteen 2-min intervals following the apomorphine challenge was counted and recorded.

Drugs

Drugs used in this investigation included apomorphine hydrochloride (Merck and Company, Rahway, NJ), naloxone hydrochloride (Endo Laboratories, Garden City, NY) and L-prolyl-L-leucyl-glycinamide (Abbott Laboratories, North Chicago, IL). Apomorphine and naloxone solutions were prepared in double distilled water. One drop of 0.1-N hydrochloric acid was added to each 10 ml of apomorphine solution as an antioxidant. PLG solutions were prepared in 0.01-N glacial acetic acid. All drugs were administered intraperitoneally in injection volumes of 0.1 ml/10 g for mice and 0.1/100 g for rats. Distilled water, naloxone and PLG pretreatments were routinely administered 5 min prior to the apomorphine challenge in both mice and rats.

Statistical Analysis of Data

In assessing the influence of naloxone and PLG pretreatments upon apomorphine-induced drug effects, the mean mouse climbing scores and the mean number of rat rotations of variously treated groups were compared, using analysis of variance and the multiple range test of Duncan. These procedures were part of an SPSS statistical package and performed on the Marquette University Sigma-9 computer system.

RESULTS

Experiments in Mice

Upon placement into the circular cages, mice initially demonstrated exploratory behavior and some mice did exhibit transient climbing activity; however, after 30 min, all animals generally became quiescent. Treatment with the glacial acetic acid solvent and/or distilled water failed to evoke any climbing activity. The administration of naloxone or PLG alone produced a slight degree of climbing activity but only at extremely high doses. A standard challenge dose of 2.0 mg/kg of apomorphine, on the other hand, typically evoked a mean stereotypic climbing score of approximately 1.2. Pretreatment of two different groups of mice with two doses of naloxone 5 min prior to the apomorphine challenge resulted in dose-related potentiation of the climbing response, yet pretreatment with two doses of PLG failed to appreciably influence apomorphine-induced climbing activity. The findings of these mouse experiments are shown in Fig. 1.

Experiments in Rats

When placed into the hemispherical bowls, some rats exhibited turning behavior; however, this activity usually subsided after 5 min or less. Treatment with distilled water, 1.0 mg/kg of naloxone or 1.0 mg/kg of PLG failed to precipitate any rotational behavior. Animals receiving a standard challenge dose of 1.0 mg/kg of apomorphine 5 min after distilled water pretreatment exhibited significant turning in a contralateral direction for more than 30 min after injection. There was no noticeable influence upon such rotational behavior exerted by either of two doses of naloxone administered 5 min prior to the apomorphine challenge; on the other hand, rats receiving PLG pretreatment showed dose-related potentiation of the contralateral turning activity. The findings of these rat experiments are shown in Fig. 2.

DISCUSSION

Research by us [14, 18, 24, 25, 26] and others [1, 8, 9, 10, 11] has shown that narcotic antagonist drug pretreatment can potentiate the effects of dopaminergic drugs, although there have also been reports of naloxone antagonism rather than potentiation of apomorphine-induced stereotypic effects [15,16]. Pretreatment with the neuropeptide PLG has also been found to enhance dopaminergic drug effects [13, 20, 21]. A definitive mechanism by which each of these drugs works is as yet undetermined.

The present research was originally designed to compare the influences and potencies of naloxone and PLG in enhancing the effects of apomorphine in two standard paradigms of striatal dopaminergic activity. Considering the reported narcotic antagonistic properties of PLG [3,7], we were also interested in whether PLG might work in a manner similar to naloxone. We discovered quite unexpectedly that there was an asymmetrical potentiation of apomorphine by naloxone and PLG in these tests. In the one paradigm, naloxone but not PLG, even at high doses, enhanced apomorphine-induced stereotypic climbing activity in mice. In the other paradigm, PLG but not naloxone, at comparable doses, increased apomorphine-induced rotational behavior in rats with unilateral 6-OH-DA lesions of the substantia nigra.

The mouse climbing test has been advanced by several laboratories as a simple and convenient measure of striatal dopaminergic receptor stimulation [5, 23, 30]. The rat rotational model is a widely used albeit more complex test system for assessing striatal dopaminergic activity [29]. The primary difference between these two paradigms is that the latter requires development of dopaminergic receptor supersensitivity on the side of the lesion subsequent to unilateral damage to the nigrostriatal pathway. When activated by apomorphine, these supersensitive receptors give rise to a unilateral dominance of striatal dopaminergic activity and the animal rotates away from the side of the lesion. There is no such functional imbalance in the mouse climbing model, wherein apomorphine presumably stimulates striatal dopaminergic receptors bilaterally in evoking stereotypic climbing activity.

The inability of naloxone to potentiate apomorphine-induced contralateral rotations in rats with unilateral

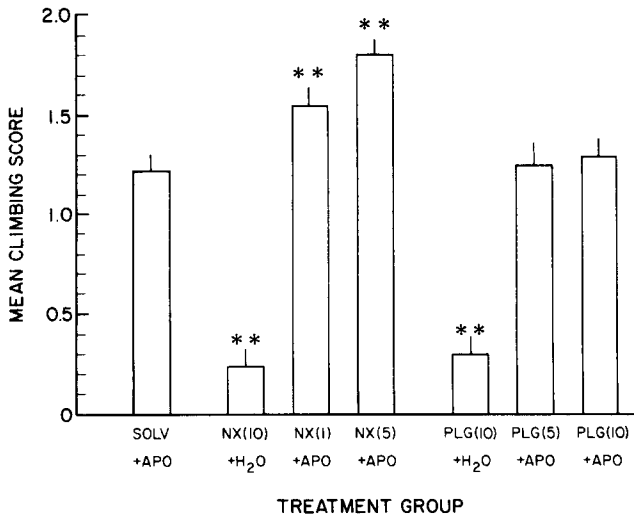

FIG. 1. Mean climbing scores of groups of mice receiving various drug pretreatments. All groups consisted of at least 20 mice. Vertical lines represent the s.e.m. Significance of difference: $^{**}p<0.01$, compared to SOLV + APO group.

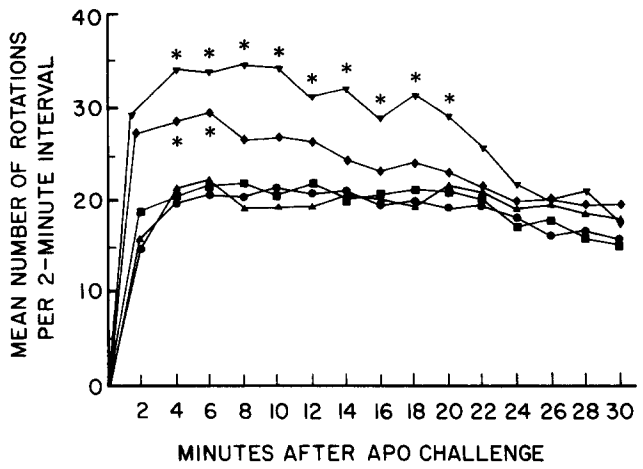

FIG. 2. Apomorphine-induced rotational behavior in unilaterally 6-OH-DA-lesioned rats pretreated with: ●, distilled water; ■, 1.0 mg/kg of naloxone; ▲, 5.0 mg/kg of naloxone; ◆, 1.0 mg/kg of PLG; and ▼, 5.0 mg/kg of PLG. Each curve represents the mean rotational behavior of 8 rats. Significance of difference: $^{*}p<0.05$, compared to distilled water-pretreated group.

6-OH-DA lesions of the substantia nigra is consistent with the findings of an earlier study [6]; however, it is incongruous with an earlier observation in which naloxone enhanced apomorphine-induced ipsilateral rotations in rats with unilateral electrocoagulation lesions of the substantia nigra [26]. This discrepancy can be attributed to the different rotational models created by chemolytic vs. electrolytic lesions of the substantia nigra. Electrocoagulation produces a more nonspecific lesion that may include nondopaminergic nerve fibers essential to rotational behavior, thus apomorphine acts upon dopaminergic receptors in the intact striatum and elicits ipsilateral turning [4].

One possible explanation for naloxone potentiation of dopaminergic drug effect is that naloxone blocks opiate receptors which are located on striatal dopaminergic nerve terminals and which are inhibitory to dopaminergic neuronal function [22,27]. It is plausible that naloxone acts in this manner to potentiate apomorphine-induced drug effects in the mouse climbing model and the electrolytic rat rotational paradigm. However, the failure of naloxone to increase apomorphine-induced turning in rats with 6-OH-DA lesions might be due to the absence of dopaminergic nerve endings and their attendent opiate receptors on the lesioned side of the brain. PLG, on the other hand, has not been found to exert any significant influence upon dopamine neurochemistry and has been suggested to perhaps act directly upon the dopaminergic receptor to enhance its sensitivity [13]. Its effectiveness in potentiation of apomorphine in the rat rotational paradigm can thus be explained, however, why PLG should fail to enhance apomorphine-induced stereotypic climbing in mice even at high doses is unclear.

It is conceptually difficult to compare the influences of two different drugs upon two different test systems in two different species. It is possible that apomorphine might produce the two behaviors by stimulation of different populations of striatal dopaminergic receptors in the two species that have different susceptibilities to naloxone or PLG intervention. It is equally plausible that naloxone and PLG have two different mechanisms of action in enhancing dopaminergic drug effects. Further research is currently in progress attempting to compare these drug interactions in various experimental paradigms and to determine test or species differences in manifestation of the potentiated drug effect.

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