Effects of Pro-Gly-Pro Tripeptide on the Dopamine System

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Tripeptide Pro-Gly-Pro interacted with dopamine receptors *in vitro* and reduced behavioral manifestations of apomorphine-induced hyperfunction of the dopamine system in verticalization, stereotypy, and yawning tests. Presumably, the behavioral effects of Pro-Gly-Pro tripeptide were mediated through post- and presynaptic D_2 and D_3 receptors.

Key Words: *glyprolines; Pro-Gly-Pro; apomorphine-induced verticalization; stereotypy; dopamine receptors*

Glyprolines is a recently discovered family of bioactive peptides consisting of glycine (Gly) and proline (Pro). One of them, Pro-Gly-Pro tripeptide, reduces blood clotting and inhibits ulcer formation in the stomach [8]; its preclinical studies have been carried out. The effects of PGP on CNS activity were demonstrated in animal experiments, primarily the stress-protective effect. The drug prevents increase in anxiety and suppression of orientation and exploratory activity of stressed rats [5]. Presumably, the direct effects of glyprolines on the CNS structures involved in the formation of response to stress play the key role in these effects. These characteristics are assumed to be essential for the realization of other effects of glyprolines, for example, their antiulcerogenic effect on the gastric mucosa under conditions of stress inducing ulcer formation [8]. In addition, PGP exhibited a neuroprotective effect *in vitro* on nerve cell culture under conditions of oxidative stress [6] and *in vivo* on hypoxic animals [9]. Presumably, the effect of PGP on hypoxic resistance is due to blood flow stimulation and more rapid saturation of tissues with oxygen as a result of glyproline preinjection. Incorporation of glyproline in the C-terminal structure of other bioactive peptides improves the resistance of the resultant molecule to proteases, stimulates and even modulates the physiological effects of the initial peptide, as exemplified by such drugs as Semax (Met-Glu-His-Phe-Pro-Gly-Pro) with marked nootropic and neurotropic effects and Selanc (Thr-Lys-Pro-Arg-Pro-Gly-Pro) anxiolytic.

Despite great variety of detected forms of biological activities of glyprolines, the neurochemical mechanism of their action received little attention. We studied the interactions of PGP with the dopamine system at the behavioral and receptor levels.

MATERIALS AND METHODS

The study was carried out on outbred male albino mice (20-25 g) and outbred male albino rats (200-250 g) from Kryukovo Breeding Center of the Russian Academy of Medical Sciences. PGP was injected intraperitoneally in 0.2 ml saline 30 min before testing.

Verticalization phenomenon was induced by injection of apomorphine (Sigma) to mice in a dose of 5 mg/kg subcutaneously directly before testing. The testing was carried out in special cylindrical boxes 140 mm in diameter with a wall from vertical stainless wires 2 mm thick and 200 mm long, with 10 mm distance between them. The level of verticalization was evaluated over 10 sec every 2 min throughout one hour. After the experiment, the summary score (number of paws on the wire wall) was calculated for each animal during the entire period of observation. The stereotypy phenomenon was induced in rats by

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apomorphine injection in a dose of 0.75 mg/kg subcutaneously directly before testing. The total duration of stereotypical behavior and intensity of stereotypical reactions were evaluated by the following score: 1 point: miscellaneous stereotypical movements (for example, accidental sniffing); 2 points: intense short stereotypy (including licking, gnawing); 3 points: constant intense stereotypy. Stereotypy level was evaluated over 60 sec every 10 min throughout one hour. The summary score for each animal was calculated. The yawning phenomenon was induced in rats by injection of apomorphine in a dose of 0.1 mg/ml subcutaneously directly before testing. The effect of the drug in this dose is directed mainly at the dopamine presynaptic receptors. The number of yawns was counted during 1 h in each animal.

Binding of ^{3}H-spiperone to rat cerebral frontal cortex was carried out in 300 μl 50 mM Tris-HCl buffer (pH 7.4) containing cell membranes (0.15 mg protein/ml), basitracin (50 μg/ml), 10 μM pargilin, and 5 nM ^{3}H-spiperone (95 Ci/mmol; synthesized and kindly offered by Prof. Yu. A. Zolotarev, Institute of Medical Genetics). The reaction mixture for spiperone binding to the striatum receptors contained additionally 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and ascorbic acid (0.1 mg/ml); the concentration of ^{3}H-spiperone was 0.7 nM. In order to evaluate nonspecific binding, unlabeled spiperone in a concentration of 1 μM was added into incubation medium. Analysis of ^{3}H-spiperone exclusion with sulpiride and PGP was carried out for concentrations from 0.1 nM to 100 μM. Incubation was carried out for 40 min at 25°C. Bound and free labels were separated on GF-B filters (Whatman) soaked in 0.1% polyethylenamine solution. Each point was evaluated 3 times in 3 independent experiments.

The results were statistically processed by the Mann–Whitney test using Statistica software.

RESULTS

The curves of ^{3}H-spiperone exclusion from sites of its specific binding to the membrane fraction of striatal cells, where the density of D_2 dopamine receptors is maximum, with sulpiride (selective ligand of these receptors) and PGP are presented in Figure 1. The curve of ^{3}H-spiperone exclusion with sulpiride reaches a plateau without attaining 50% binding level. This indicates the presence of receptors other that D_2 in the striatum binding spiperone. The maximum exclusion of ^{3}H-spiperone with PGP reaches 90%, 50% exclusion being attained at peptide concentration of 9 nM. Hence, PGP presumably interacts not only with D_2, but also with some other spiperone receptors and its affinity for them is by an order of magnitude lower than

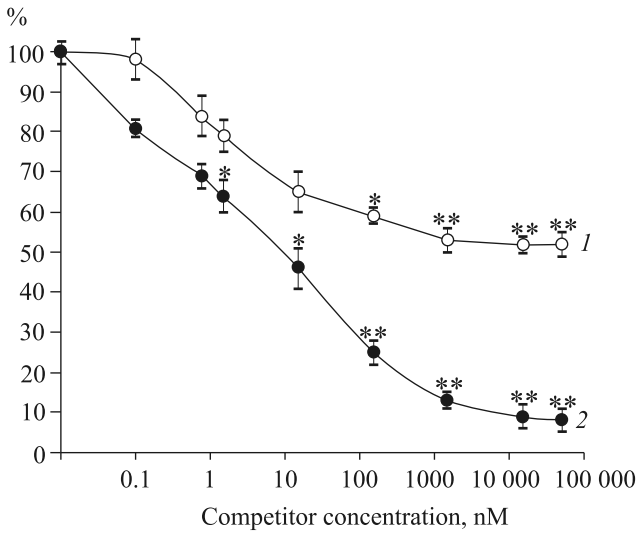

Fig. 1. Effects of PGP on specific binding of ^{3}H-spiperone to cell membranes in rat striatum. Ordinate: specific binding. *1*) sulpiride; *2*) PGP. **p*<0.05, ***p*<0.01.

that of spiperone. Spiperone is a nonselective ligand of 5-HT_1 and 5-HT_2 serotonin receptors, D_2 dopamine receptors (D_2, D_3, D_4), and of α_1 adrenoceptors. In our study under conditions optimal for ^{3}H-spiperone reactions with 5-HT_2 receptors in the frontal cortex (the brain site with the highest density of these receptors [10]), PGP in concentrations up to 100 μM virtually did not modify binding of ^{3}H-spiperone. Independent experiments also showed that tritium-labeled PGP specifically bound to the basal nuclear membranes, but not to other compartments of rat forebrain [4]. The results suggest that PGP reacts with D_2 dopamine receptors and adrenoreceptors. This is confirmed by the data on the capacity of PGP and its fragments to reduce the tone of the aorta, elevated by norepinephrine [2].

PGP in a dose of 10 mg/kg reduced behavioral manifestations of drug-induced hyperactivation of the dopamine system *in vivo*. This effect was observed in the verticalization test reflecting the increase of dopaminergic transmission in the cerebral mesolimbic structures induced by injection of high doses of apomorphine (5 mg/kg; Table 1). Apomorphine in a medium dose (0.75 mg/kg) stimulated dopaminergic transmission in the nigrostriatal system, thus causing stereotypy. Under these conditions PGP in a dose of 10 mg/kg decreased the duration and number of stereotypical movements in rats by 20% (Table 1).

It is assumed that psychomimetics stimulate motor and stereotypical activities of experimental animals through postsynaptic D_1 and D_2 dopamine receptors, while D_3 receptor stimulation inhibits locomotion [12,13]. On the other hand, it was shown that the yawning phenomenon is primarily caused by stimulation of presynaptic D_3 receptors, while D_2 autorecep-

TABLE 1. Effects of PGP on Behavioral Manifestations of Dopamine System Stimulation Caused by Apomorphine Injection

PGP dose, mg/kg	Verticalization, score	Stereotypical movements	Yawns	
			intensity, score	duration, min
0	79±5	2.44±0.06	69±2	12±1
0.001	80±6	—	—	—
0.01	77±5	—	—	—
0.1	74±7	—	—	—
1	69±6	2.29±0.08	62±3	12±2
10	62±5*	2.01±0.10**	56±3**	8±1*

Note. *$p<0.05$, **$p<0.05$ compared to the control.

tors mediate its inhibition [11]. The PGP reduced the number of yawns by 35% in rats (Table 1), this indicating blocking of presynaptic D_3 receptors. Hence, our study detected the PGP capacity to react with D_2 dopamine receptors *in vitro* and block apomorphine reactions with post- and presynaptic dopamine receptors of D_2 and D_3 types *in vivo*. Opposite effects mediated by these receptors can be responsible, along with other factors, for just 20-30% realization of behavioral effects of PGP.

Very low efficiency of PGP *in vivo* can be also due to its pharmacokinetics. It enters unchanged into the circulation and crosses the blood-brain barrier [3]. However, just one thousandth of the injected peptide reaches the brain and is gradually hydrolyzed with the formation of GP as the main metabolite [1]. The neurotropic effects of this dipeptide on stressed animals are no less pronounced than the effects of PGP [5]. GP is the main metabolite of Selanc, a synthetic peptide anxiolytic [1]. We also observed a depriming effect of Selanc in verticalization test, but this peptide did not modify ^{3}H-spiperone binding to D_2 receptors *in vitro* [7]. Summing up these data, we think that not only PGP, but also its GP fragment are characterized by dopamine-blocking effects.

It is assumed that the development of positive symptoms in schizophrenia is associated with hyperactivation of the dopamine system, while blocking of dopamine receptors is the main mechanism of pharmacological effect of neuroleptics. PGP unites the characteristics of a D_2 receptor antagonist with the neuroprotective [6,9] and nootropic [5] effects, and hence, can be regarded as a candidate antipsychotic drug effective for therapy of positive symptoms and cognitive disorders in schizophrenia and other psychoses.

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