Anxiolytic Activity of Endogenous Nootropic Dipeptide Cycloprolylglycine in Elevated Plus-Maze Test

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Testing in an elevated plus-maze revealed dose-dependent anxiolytic activity of piracetam analog cycloprolylglycine. Intraperitoneal injection of this agent (0.05 mg/kg) 9-fold prolonged the time spent in open arms compared to the control, without affecting the total motor activity. This effect was stereo-selective: D-enantiomer in doses of 0.05 and 0.1 mg/kg was inactive. Therefore, cycloprolylglycine is similar to piracetam in not only nootropic, but also anxiolytic activity. The existence of an endogenous system responsible for the co-regulation of memory and anxiety is hypothesized.

Key Words: *cycloprolylglycine; anxiolytic activity; elevated plus-maze*

Cycloprolylglycine (CPG) is a highly active nootropic peptide constructed as a topological analog of classical nootropic piracetam [3] on the assumption on peptidergic mechanisms of the effect of piracetam. CPG was identified by chromatographic mass spectrometry as an endogenous compound in rat brain [9].

We previously showed that CPG is similar to piracetam by its mnemotropic effects: when administered before training, it prevented the development of retrograde amnesia in the conditioned passive avoidance test, injection of this agent immediately after training was ineffective, while administration before retrieval of the habit potentiated amnesia [4]. In the model of active avoidance, CPG increased the number of rats approaching the training criterion, although the number of presentations needed to comply this criterion remained unchanged [10]. Similar to piracetam, CPG increased the amplitude of transcallosal evoked potential in rat brain without modifying its shape [10].

It was recently shown that apart from its nootropic activity, piracetam produces also an anxiolytic effect. In the J. Vogel conflict test, piracetam (400-600 mg/kg) demonstrated anxiolytic activity and significantly increased the number of punished drinking in rats [2]. Piracetam administered in a dose of 250 mg/kg for 7 days produced an anxiolytic effect in the elevated plus-maze (EPM) test [5]. Anxiolytic effect of piracetam was also observed in clinical practice [1].

These findings prompted us to elucidate whether CPG, an endogenous peptide analog of piracetam, possesses similar properties. To this end, EPM test, the most adequate tools for studying anxiolytic activity was used [6,7,11]. Stereoselectivity of the anxiolytic effect produced by CPG was also examined.

MATERIALS AND METHODS

Experiments were carried out on outbred albino male rats (n=108) weighing 200-270 g. The animals were kept in standard plastic cages (60×40×20 cm, 8 rats per cage) and were given granulated food and water *ad libitum*. Each rat was subjected to handling for 3 days before the experiments. Three-four hours before the experiments the rats were placed in experimental room. Tests were carried out at 16.00-20.00 p.m. in a dark room; the light source (60 W bulb) was screened in such a way that the setup was illuminated only by reflected light. CPG enantiomers were dissolved *ex*

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tempore and injected intraperitoneally 15 min before maze testing. The control rats received equivalent volume of distilled water. Peptides were injected in the following doses (mg/kg): L-CPG (0.05, 0.1, 0.2, and 1.0) and D-CPG (0.05 and 0.1). Basic modification of EPM was used [11]: 4 arms (50 cm in length and 15 cm in width; the height of sides in dark arms was 15 cm) and central start platform (15×15 cm). The maze was elevated by 75 cm. The floor in each arm was covered with black enamel and divided into two equal parts by a white line. To evaluate motor activity, the number of crossing of this line and the line separating the arm and start platform was counted. The rat was placed in the center of the maze and its behavior was recorded for 5 min. The following parameters were evaluated: total horizontal motor activity in open and dark arms; vertical motor activity (rearings); the total number of entries into all arms; the number of entries into open arms (absolute value and percentage of the total number); the number of entries into dark arms; time spent in open arms (absolute time and percentage of the total time of testing), the number of entries and the time spent on the central platform.

The data were averaged within the groups and analyzed statistically using non-parametrical Mann—Whitney *U* test at $p<0.05$ and Complete Statistical System (Version B640) software.

RESULTS

Intraperitoneal injection of L-CPG (0.05 mg/kg) significantly increased the numbers of entries into open arms by 2.8 times (Table 1; Fig. 1, *a*). The relative number of entries into open arms increased 5.5-fold, the absolute and relative duration of stay in open arms increased 9-fold. There is evidence that the increase in the number of entries and duration of stay in the open arms attests to anxiolytic activity, while the relative parameters characterize the selectivity of the test anxiolytic preparation [6,11,12]. In a dose of 0.1 mg/kg, L-CPG increased the absolute and relative numbers of entries into open arms by 9 and 4.5 times, respectively. The absolute and relative duration of stay in open arms increased 6.5-fold. In a dose of 0.2 mg/kg the drug the drug was less effective and only a tendency to increase in the above parameters was noted. In a dose of 1 mg/kg the test drug produced no anxiolytic effects.

L-CPG in doses of 0.05, 0.2, and 1 mg/kg did not enhanced motor activity (the effect characteristic of psychostimulator preparations [13]), but in a dose of 0.1 mg/kg the preparation induced a significant (2-fold) increase in motor activity.

Parameters of exploratory activity (the number of entries into all arms and the central platform) and vertical motor activity significantly surpassed the corresponding control values only when CPG was injected in the dose of 0.1 mg/kg. The increase of these indices attests to nootropic properties of the peptide similar to those of piracetam [8]. It is noteworthy that CPG in this dose exhibited nootropic activity in various behavioral and electrophysiological tests.

When administered in doses of 0.05-0.1 mg/kg, D-enantiomer was ineffective (Fig. 1, *b*), which confirmed stereoselectivity of the described effects of L-CPG.

Therefore, when applied in doses of 0.05 and 0.1 mg/kg, L-CPG induced pronounced increase in the behavioral indices associated with anxiolytic activity in EPM test; the effect of L-CPG in a dose of 0.05 mg/kg was most specific. When used in a dose of 0.1 mg/kg, CPG demonstrated also psychostimulatory and nootropic effect in addition to its anxiolytic action (Fig. 1, *a*).

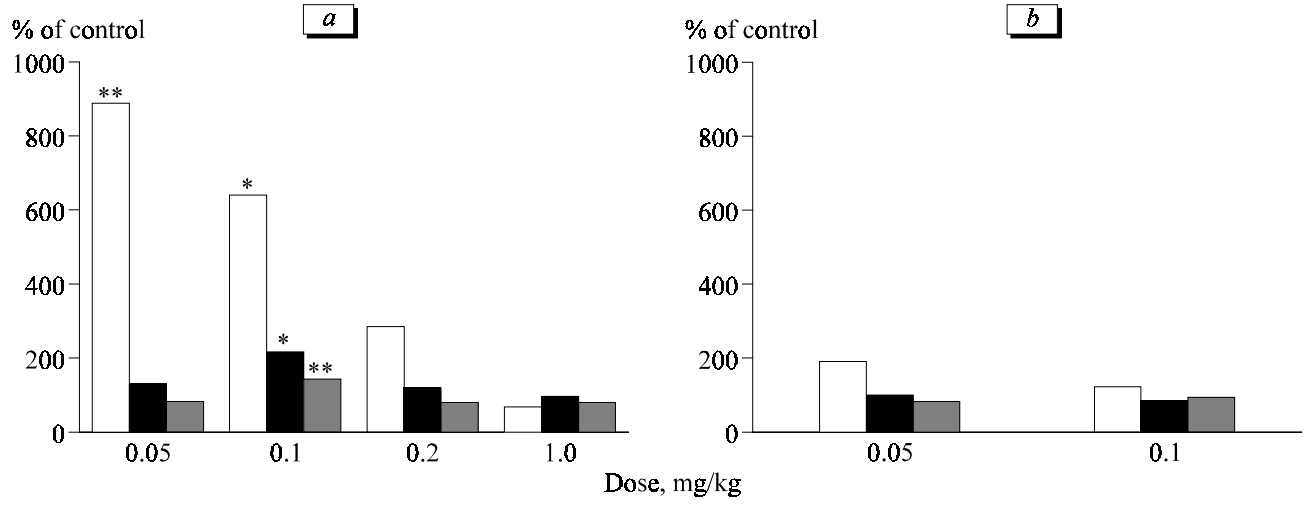

Fig. 1. Effects of cyclo-L-prolylglycine (*a*) and cyclo-D-prolylglycine (*b*) on rat behavior in elevated plus-maze. Open bars: relative time spent in open arms; dark bars: horizontal motor activity; hatched bars: vertical motor activity. *$p<0.01$, and **$p<0.05$ compared to the control.

TABLE 1. Effect of CPG on Rat Behavior in EPM (Mean Indices)

Index	Control 1 (*n*=17)	CPG, mg/kg		Control 2 (*n*=12)	CPG, mg/kg	
		0.1 (*n*=19)	1.0 (*n*=8)		0.05 (*n*=10)	0.2 (*n*=10)
Number of entries						
total	2.35	6.1*	2.75	3.08	5.30	4.80
into open arms						
abs.	0.20	1.79*	0.25	0.58	1.60***	1.50
%	6.47	29.1**	4.59	5.86	32.2***	23.9
Time spent in open arms, sec						
abs.	2.15	13.8*	1.46	6.38	57.4***	19.3
% of total	0.72	4.61**	0.49	2.15	19.1***	6.12
Number of entries into dark arms	2.18	4.26***	2.50	2.50	3.70	3.20
Number of entries to central platform	0.80	3.90*	–	1.25	2.70	1.80
Time spent on central platform, sec	5.00	29.4**	–	8.54	23.7	17.9
Motor activity, arb. units						
horizontal	7.88	17.1**	7.63	12.3	16.1	14.6
vertical	8.41	12.1***	6.80	12.1	9.90	9.70

Note. *p<0.005, **p<0.01, and ***p<0.05 compared to the control.

Low efficective doses of CPG and stereoselectivity suggest that its anxiolytic effect is mediated via a receptor mechanism. The receptors mediating the effect of L-CPG differ from those mediating its nootropic effect, since doses producing maximum nootropic and anxiolytic effects were different. Similar feature is characteristic of piracetam, but in this case the nootropic doses are lower than anxiolytic ones [2]. Nootropic and anxiolytic activities of endogenous dipeptide suggest the existence of a system coregulating memory and anxiety.

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