PHARMACOLOGY AND TOXICOLOGY

Endogenous Dipeptide Cycloprolylglycine Shows Selective Anxiolytic Activity in Animals with Manifest Fear Reaction

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Experiments on two mouse strains with opposite reactions to emotional stress showed selectivity of the anxiolytic effect of endogenous dipeptide cycloprolylglycine. In the open field test cycloprolylglycine (0.01-0.10 mg/kg intraperitoneally) dose-dependently (1.8-2.1-fold) increased motor activity of BALB/c mice with manifest fear reaction and had no effect on C57Bl/6 mice with active behavior. The content of endogenous cycloprolylglycine in mouse brain correlated with the type of emotional stress reaction: its content in the brain of C57Bl/6 mice 1.5 times surpassed that in BALB/c mice. It is concluded that cycloprolylglycine is involved in the endogenous regulation of fear reaction.

Key Words: *cycloprolylglycine; content in mouse brain; anxiolytic activity; BALB/c; C57Bl/6*

Endogenous dipeptide cycloprolylglycine was previously identified in rat brain [7]. In standard psychopharmacological experiments synthetic analog of this dipeptide injected intraperitoneally in doses of 0.1-1.0 mg/kg produced a mnemotropic effect [4,8], while in doses of 0.05-0.10 mg/kg it possessed anxiolytic activity [3]. It is therefore important to evaluate the anxiolytic effect of this compound in animals with different phenotypes of fear reaction to emotional stress.

We measured the content of endogenous cycloprolylglycine in the brain of C57Bl/6 and BALB/c mice. C57Bl/6 mice are characterized by active behavior in the open field test with predominating exploratory activity, while BALB/c mice demonstrated an opposite reaction: reduced motor activity and increased number of defecations, which indicated pronounced fear reaction [5,10]. The effect of cycloprolylglycine on animals behavior in the open field test was studied on these experimental models.

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MATERIALS AND METHODS

Experiments were carried out on C57Bl/6 and BALB/c mice (20-22 g) from Stolbovaya Breeding Center of the Russian Academy of Medical Sciences. The animals were kept in a vivarium, 10 per cage, on standard diets with free access to water and normal 12-h day/night regimen. All tests were performed from 9.00 to 13.00.

Cycloprolylglycine (synthesized at the Department of Chemistry, Institute of Pharmacology, Russian Academy of Medical Sciences [2]) was dissolved in distilled water and injected intraperitoneally in doses of 0.01-0.10 mg/kg (1 ml). Controls were injected with 1 ml distilled water.

Open field testing was performed using a light flash [1]. Fifteen minutes after cycloprolylglycine injection the animals were kept in darkness for 1 min and then placed in a peripheral sector of the open field (white round arena, 1 m in diameter, with 50-cm white walls evenly illuminated with four 75-W bulbs at a height of 1 m). Four concentric circles on the arena were divided into sectors so that the peripheral circle

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TABLE 1. Effects of Cycloprolylglycine in Different Doses (mg/kg, Intraperitoneally) on Open Field Behavior in Mice ($M \pm m$, n=8-11)

Motor activity	BALB/c			C57Bl/6		
	0	0.01	0.1	0	0.01	0.1
Peripheral	36.0±3.7	63.3±5.7*	75.7±5.0*	87.2±4.9	84.3±8.9	80.8±8.6
Central	1.6±0.9	3.3±1.2	1.4±0.8	24.1±4.9	32.7±4.9	36.7±6.0
Vertical	0.0±0.0	0.0±0.0	0.0±0.0	6.6±1.6	11.0±2.5	3.6±1.2
Total	37.6±3.9	66.5±6.3*	77.1±4.9*	118.7±8.0	129.0±10.7	122.3±14.2

Note. *p<0.01 compared to the control (dose 0).

consisted of 16 similar sectors. The number of crossed peripheral (peripheral activity) and central segments and excursions into the center (central activity), and number of rearings were counted for 3 min. The total motor activity was calculated as the sum of all types of motor activity.

The content of endogenous cycloprolylglycine in the brain was measured by high-pressure liquid chromatography (Fig. 1).

The mice were decapitated, the brain was removed, washed in cold normal saline, weighed, and homogenized in ice-cold distilled water (1:2 v/v) in a glass homogenizer for 1 min. The samples were shaken for 10 min with 3 volumes of acetonitrile and centrifuged at 8000 rpm for 10 min. Supernatants were dried in nitrogen flow at 18-20°C, the sediments were dissolved in 2 ml distilled water and analyzed by chromatography (200-µl fractions, 25°C) on a Nucleoril C-18 column (250×4.6 mm, 5-µ graining) in an acetonitril-10 mM potassium orthophosphate (pH 3.2)-water (1:65:34) system on a Perkin Elmer 250 chromatograph with Perkin Elmer LC-290 UV detector. The elution time was 14 min at a flow rate of 1 ml/min. Nine mice of each strain were used.

The cycloprolylglycine concentrations in mouse brain were processed using Student's t test and the results of behavioral tests were processed using Newman—Keuls test. The differences were significant at p<0.05.

RESULTS

Brain content of endogenous cycloprolylglycine in two mouse strains was different: 72.99±14.59 (51.04-101.38) nmol/g wet brain tissue in C57Bl/6 and 49.46±9.97 (35.89-68.71) nmol/g in BALB/c mice (p<0.05).

Injections of cycloprolylglycine in doses of 0.01 and 0.1 mg/kg to C57Bl/6 mice did not change their behavior in the open field test (Table 1). In BALB/c mice cycloprolylglycine in a dose of 0.01 mg/kg 1.8-fold increased motor activity in comparison with the control. Increasing the dose to 0.1 mg/kg increased motor activity to 210% (Table 1). Hence, this compound prevented fear reaction in BALB/c mice.

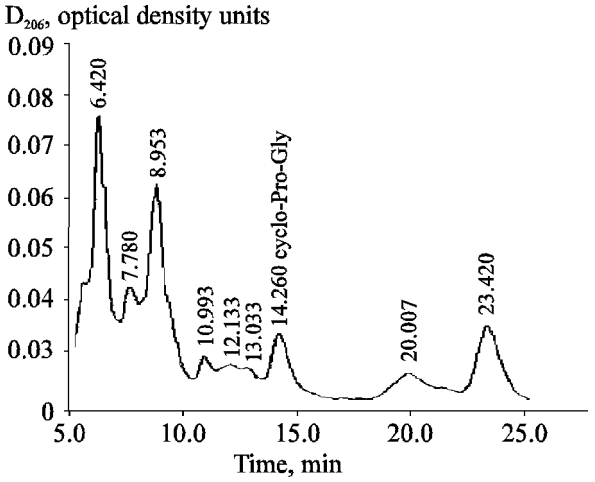

Fig. 1. Typical chromatogram of acetonitrile extract of mouse brain (HPLC).

This effect of cycloprolylglycine is similar to the effects of selective anxiolytics afobazole [6] and GV150013 [9] improving open-field behavior of BALB/c but not C57Bl/6 mice. On the other hand, typical benzodiazepine tranquilizers in similar experiments stimulated behavior of BALB/c mice only in a narrow range of doses and caused sedation in C57Bl/6 mice in the same doses. This latter effect determines their inefficiency in long-term drug therapy.

It can be assumed that cycloprolylglycine possesses selective anxiolytic properties. The correlation between cycloprolylglycine concentration in the brain (high in C57Bl/6 and low in BALB/c mice) and the type of animal reaction to emotional stress is obvious. This fact suggests that cycloprolylglycine is involved in endogenous regulation of fear reaction.

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