

Protective and Therapeutic Effects of Glyprolines in Psychoemotional Stress Induced by Cholecystokinin-4 Injection

S. E. Edeeva, G. N. Kopylova, Z. V. Bakaeva,
G. E. Samonina, B. A. Umarova, and A. A. Guseva

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Experiments on outbred albino male rats showed that psychoemotional stress induced by intraperitoneal injection of cholecystokinin-4 (100 $\mu\text{g/kg}$) increased anxiety, impaired orientation and exploration activities in the elevated plus-maze and hole-board tests, and increased the level of depression of Porsolt test. Preliminary intranasal administration of glyprolines (15 min before cholecystokinin) in a dose of 3.7 $\mu\text{mol/kg}$ prevented the development of stress-induced behavioral disturbances. Administration of peptides 30 min after cholecystokinin-4, *i.e.* to rats with developed behavioral disturbances, almost completely abolished these disturbances.

Key Words: *stress; cholecystokinin-4; behavior; glyprolines*

Short di- and tripeptides containing glycine and proline can cross the blood-brain barrier [3,4] and produce protective and therapeutic effects in behavioral disturbances caused by physical strain in rats [1,2,6].

Peptides Pro-Gly-Pro- and Gly-Pro produce the most pronounced effects. Preliminary intraperitoneal or intranasal administration of these peptides in doses of 3.7 and 37 $\mu\text{mol/kg}$ considerably prevented the development of post-stress behavioral disturbances, while administration of these peptides against the background of developed post-stress behavioral disturbances almost completely abolished them [1,2].

Here we evaluated the capacity of Pro-Gly-Pro and Gly-Pro to prevent and abolish post-stress behavioral disturbances in rats with experimental psychoemotional stress caused by injection of cholecystokinin-4 (CCK-4) inducing anxiety and panics in humans and animals [8-12].

MATERIALS AND METHODS

Experiments were carried out on outbred albino male rats weighing 200-250 g maintained under standard vivarium conditions with free access to food and water. CCK-4 was injected intraperitoneally in a dose of 100 $\mu\text{g/kg}$ in a volume of 0.5 ml/200 g body weight. The peptides were administered intranasally in a dose of 3.7 $\mu\text{mol/kg}$ in a volume of 0.5 ml/200 g body weight. Control animals received the same volume of physiological saline instead of the test peptides.

The peptides were synthesized in Institute of Molecular Genetics, Russian Academy of Sciences.

The behavioral parameters in the hole-board and elevated plus-maze tests were evaluated 1 h after injection of CCK-4 [2]. The time of observation was 3 min in each test.

The level of depression was evaluated using Porsolt's test. Two hours after injection of CCK-4, the animal was put in a tank with water (23°C) and the following parameters were recorded over 10 min: total duration of active swimming (active

Department of Human and Animal Physiology, Biological Faculty of M. V. Lomonosov Moscow State University. **Address for correspondence:** edeeva_saglara@mail.ru. S. E. Edeeva.

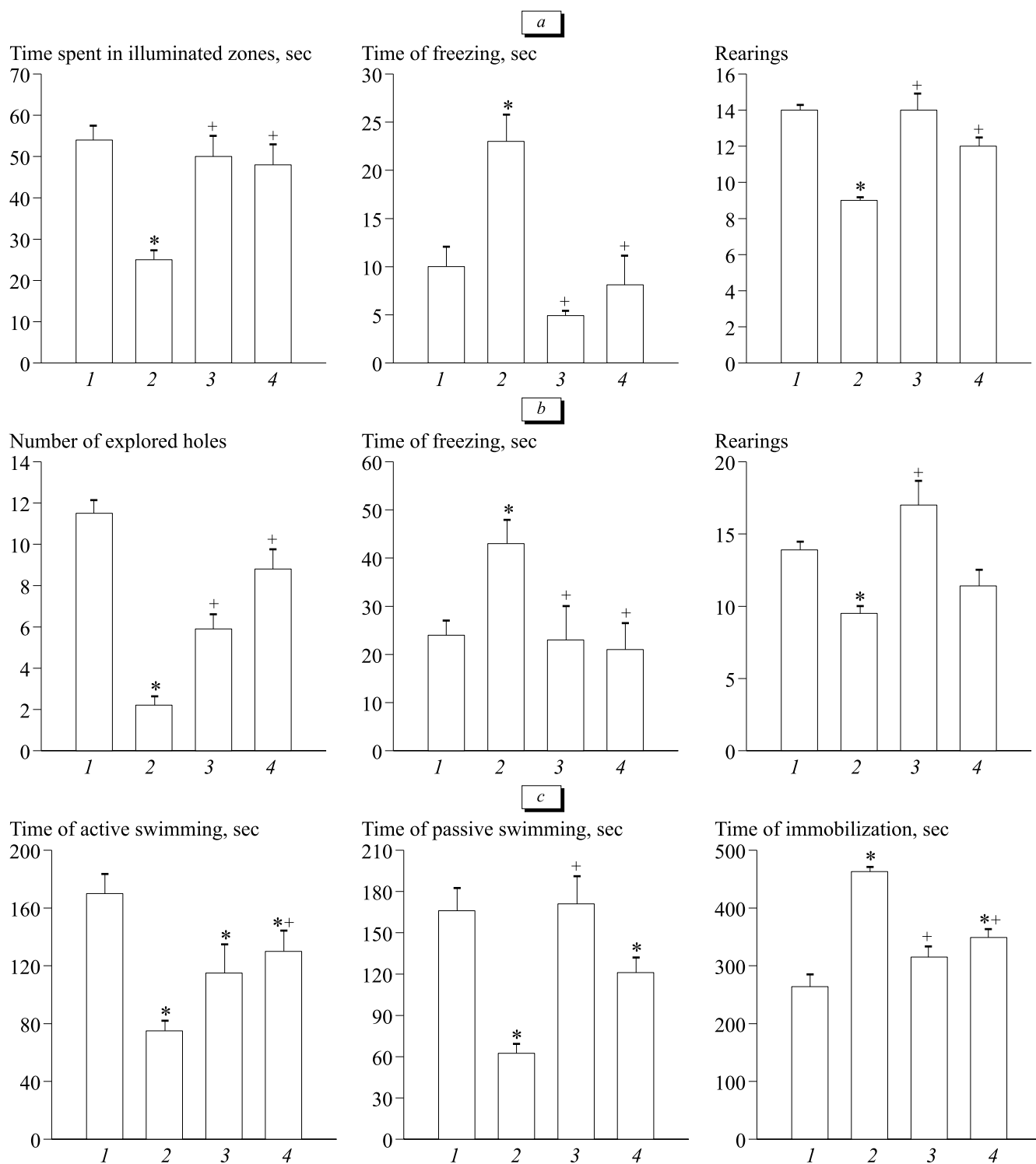


Fig. 1. Protective effects of Pro-Gly-Pro and Gly-Pro in rats with stress-induced behavioral disturbances caused by CCK-4 in elevated plus-maze (a) and hole-board (b) tests and in Porsolt test (c). 1) control ($n=54$), 2) CCK-4 ($n=25$), 3) administration of Pro-Gly-Pro 15 min before CCK-4 ($n=14$), 4) administration of Gly-Pro 15 min before CCK-4 ($n=10$). Here and on Figs. 2, 3: $p<0.05$ compared to: *control, ⁺CCK-4.

swimming movements, motion within the tank); total duration of passive swimming (rare limb and tail movements to keep the head above water surface), duration of immobility (the animal is immo-

bile and only the nose remains above the water surface).

The experiments were carried out in accordance with Rules for Conducting Animal Experiments

(order No. 742 of Ministry of Higher Education, November 13, 1984).

The data were processed statistically using ANOVA software (LSD and nonparametric Mann—Whitney tests).

RESULTS

One hour after injection of CCK-4, typical post-stress changes in animal behavior were noted: the time spent in illuminated zones decreased, the num-

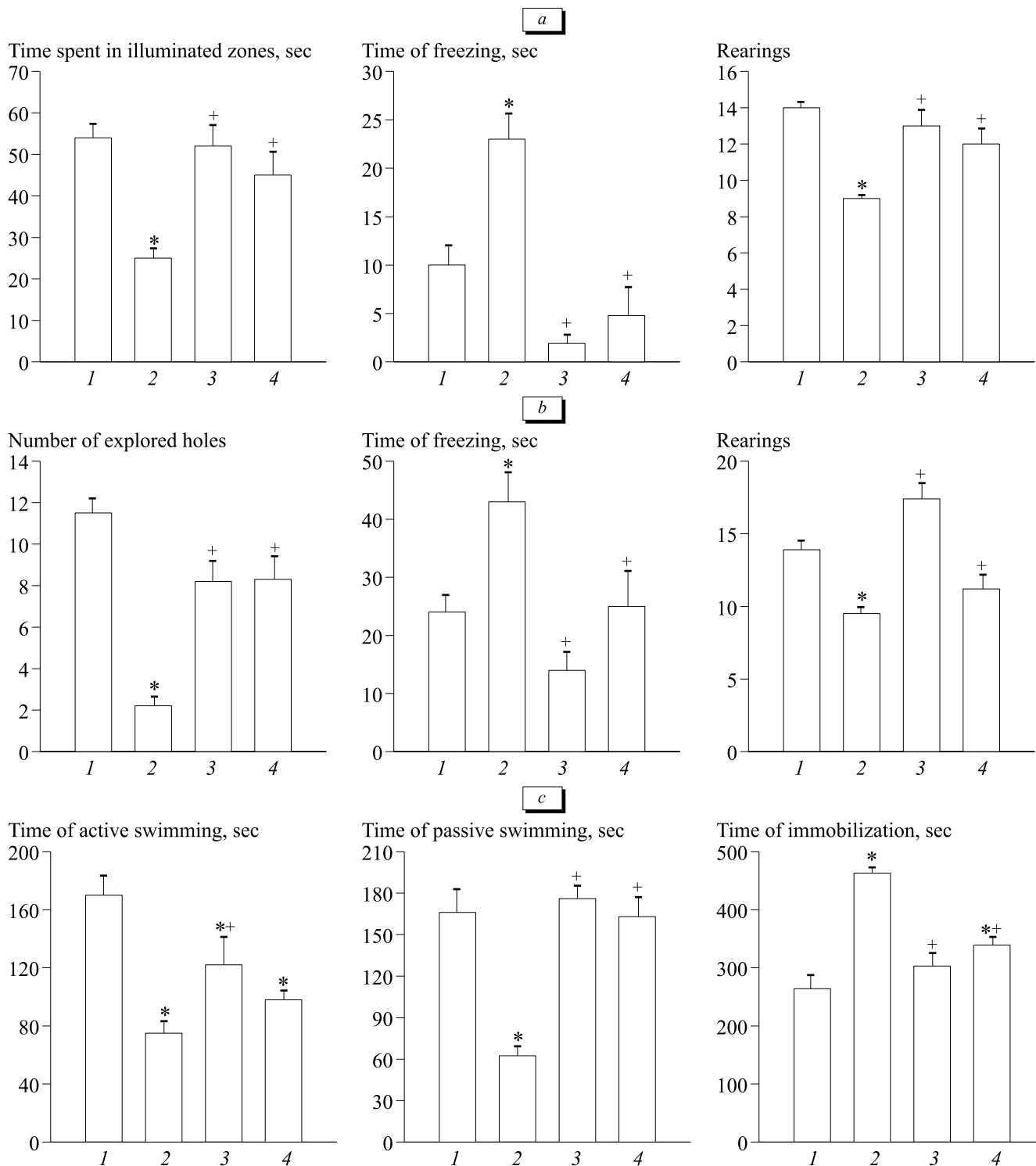


Fig. 2. Therapeutic effects of Pro-Gly-Pro and Gly-Pro in rats with stress-induced behavioral disturbances caused by CCK-4 in elevated plus-maze (a) and hole-board (b) tests and in Porsolt test (c). 1) control ($n=54$), 2) CCK-4 ($n=25$), 3) administration of Pro-Gly-Pro 30 min after CCK-4 ($n=14$), 4) administration of Gly-Pro 30 min after CCK-4 ($n=10$).

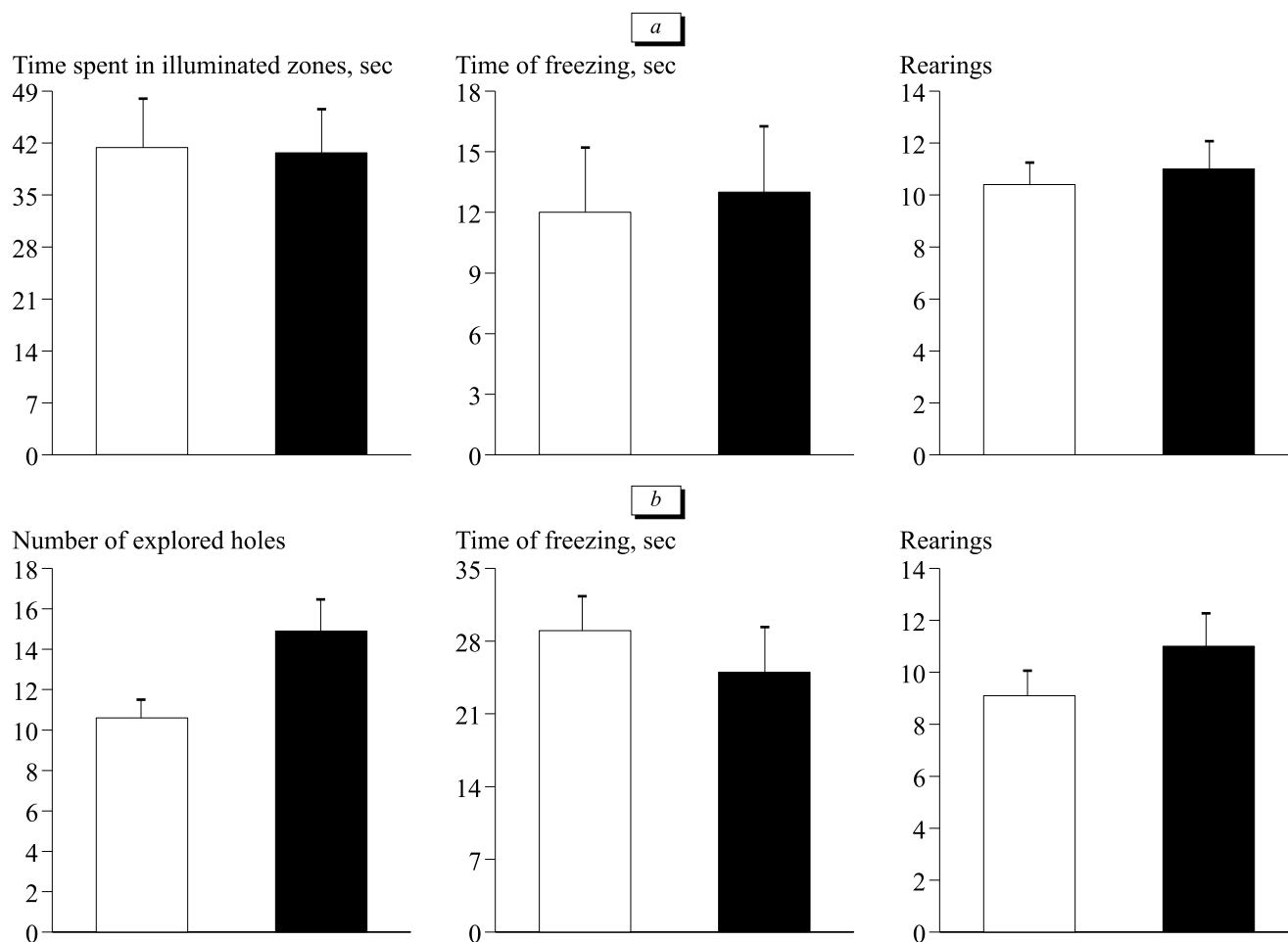


Fig. 3. Effect of Pro-Gly-Pro on elevated plus-maze (a) and hole-board (b) behavior of intact rats. Light bars: control ($n=14$), dark bars Pro-Gly-Pro ($n=13$).

ber of rearing postures and the number of explored holes decreased, while the time of freezing increased. These changes were similar to post-stress behavioral changes after physical strain (inescapable 10-min swimming) observed in our previous experiments [1,2] and attested to increased anxiety and reduced orientation and exploration activity. Moreover, increased level of depression was noted in Porsolt test, which manifested in shortening of periods of active and passive swimming, and lengthening of the immobilization time.

Preliminary intranasal administration of glyprolines Pro-Gly-Pro and Gly-Pro in a dose of $3.7 \mu\text{mol/kg}$ 15 min before CCK-4 prevented the development of stress-induced behavioral disturbances (Fig. 1): all parameters after injection of CCK-4 did not differ from normal. The efficiency of Pro-Gly-Pro and Gly-Pro was similar.

Thus, peptides Pro-Gly-Pro and Gly-Pro produce a protective (preventive) effect and prevent the development of changes in animal behavior caused by psychoemotional stress.

Published data suggest that stress-induced behavioral changes form as soon as 30 min after injection of CCK-4 [13,14]. Administration of Pro-Gly-Pro and Gly-Pro in the same doses 30 min after CCK-4 almost completely abolished the stress-induced changes and all parameters returned to normal (Fig. 2). The therapeutic effects of Pro-Gly-Pro and Gly-Pro were similar.

These data suggest that Pro-Gly-Pro and Gly-Pro exhibit therapeutic activity and normalize behavioral activity of animals disturbed due to psychoemotional stress caused by CCK-4 injection.

Administration of Pro-Gly-Pro had practically no effect on behavioral parameters in intact (not stressed) animals (Fig. 3), which agrees with previous findings [5,7].

Thus, peptides Pro-Gly-Pro and Gly-Pro can prevent the development of stress-induced behavioral disturbances and abolish formed disturbances in animal behavior caused by physical strain or psychoemotional stress, but have no effect on the behavior of animals not subjected to stress, which

dictates their further investigation as preparations eliminating negative effects of stress.

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