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Anxiolytic Effects of Gly-His-Lys Peptide and Its Analogs

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Intraperitoneal administration of tripeptide Gly-His-Lys to male rats in doses of 0.5, 5, and 50 μg/kg 12 min before the start of the experiment produced an anxiolytic effect in the elevated plus maze test manifested in an increase in the time spent in open arms and shortened time spent in the closed arms. The anxiolytic effect was most pronounced after injection of 0.5 μg/kg peptide and decreased with increasing the dose of the peptide. Replacement of L-lysine with D-lysine in the tripeptide molecule was accompanied by a significant weakening of the neurotropic effects in all studied doses. Attachment of D-alanine to N- or C-terminus of Gly-His-Lys peptide leveled its anxiolytic action in all doses; significant changes in some measures of increased anxiety after administration at 50 μg/kg were found.

Key Words: *regulatory peptides; anxiolytic effect; anxiety; behavior*

Numerous studies have demonstrated the important role of peptidergic mechanisms in the regulation of various body functions. Multifunctional nature of the effects was revealed for a number of regulatory peptides [1,5], for instance, Gly-His-Lys has a modulating effect on cell growth and differentiation [9], promotes wound healing by stimulating collagen synthesis by fibroblasts [8], and affects physiological and reparative regeneration of the liver [6]. The stimulating effect of the peptide on hair growth [10] and anti-inflammatory effect in damaged tissues [7] are also known. However, there are no data on neurotropic effects of this peptide.

In view of the prospects of using regulatory peptides for the correction of anxiety and fear [4], it seemed appropriate to study the effect of Gly-His-Lys peptide and its analogues on these forms of animal behavior.

MATERIALS AND METHODS

The study was performed on 140 male Wistar rats weighing 180 to 220 g. The rats were kept in cages (5 animals per cage) under standard vivarium conditions with free access to food and water in 12:12 light:dark regimen and controlled temperature (22± 2°C). All animals were handled before the study. Experiments were carried out between 09.00 and 14.00 h.

We used Gly-His-Lys peptide (experimental series I) and its modified analogs Gly-His-D-Lys, D-Ala-Gly-His-Lys, and Gly-His-Lys-D-Ala (experimental series II) synthesized in the Research Institute for Chemistry, Saint Petersburg State University. The peptides were dissolved in saline and administered intraperitoneally 12 min before the experiment in doses of 0.5, 5, and 50 μg/kg. Controls in both series received equivalent volumes of saline (1 ml/kg body weight).

Anxiolytic effects of the peptides were studied using the elevated plus maze (EPM) test [3]. The maze consisted of four perpendicular arms (two opposite open arms without the walls and two closed arms with walls of 30 cm height) measured 50 cm long by 14 cm wide and was elevated by 50 cm above the floor. At the beginning of the experiment, the rat was placed in the center of the maze with its head directed toward an open arm; the time spent in the open and closed arms and central area and the number of entries into the open and closed arms were recorded over 5 min. Anxiolytic effects of peptides were evaluated by the increase in the number of entries into the open arms and the time spent there.

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The animals were maintained according to the guidelines of the Helsinki Declaration on the humane treatment of animals and in accordance with the decision of the Regional Ethics Committee.

Significance of the results was evaluated by Student's *t* test.

RESULTS

Administration of Gly-His-Lys peptide in all specified doses has a marked effect on the examined behavioral responses of rats (Table 1). The maximum effect was observed at a dose of 0.5 μg/kg: the time spent in open arms increased by 136% ($p<0.01$), the number of entries into open arms, by 208% ($p<0.01$), and the time spent on the central platform, by 109% ($p<0.05$). Increasing the peptide dose to 5 μg/kg was not accompanied by enhancement of the anxiolytic action, and the majority of the studied parameters were similar to those recorded in the previous group.

Further increase in the injected dose of Gly-His-Lys to 50 μg/kg attenuated these effects and appearance of significant differences between studied parameters in the experimental groups. Thus, the time spent in open arms did not substantially differ from the control values and was significantly lower than at lower and medium doses (by 45 and 39% respectively at $p<0.05$). Moreover, the time spent in closed arms and the number of entries into closed arms in this group were lower than after administration of the peptide in a dose of 0.5 μg/kg: by 28% ($p<0.05$) and 37% ($p<0.05$), respectively. Only the peptide dose of 50 μg/kg significantly increased the number of entries into closed arms (by 45%; $p<0.05$) in comparison with the control. This phenomenon can be explained by the increase in motor activity of rats associated with increasing the dose of the peptide.

The results of the study of neurotropic effects of Gly-His-Lys prompt us to study behavioral effects of its modifications. Replacement of L-lysine with D-lysine led to a significant reduction in behavioral activity of rats and practically leveled the anxiolytic action of the peptide (Table 2). Only the time spent on the central platform after peptide injection in a dose of 0.5 μg/kg (by 134%; $p<0.01$) and 50 μg/kg (by 56%; $p<0.05$) and the number of entries into closed arms at the lesser dose (by 59%; $p<0.05$) significantly surpassed the control values.

Attachment of D-alanine to N-terminus of the Gly-His-Lys molecule did not significantly affect the studied behavioral parameters of the peptide in doses of 0.5 and 5 μg/kg. Administration of the peptide in the maximum dose (50 μg/kg) significantly reduced the time spent in open arms (by 66%; $p<0.05$) and on central platform (by 48%; $p<0.05$); the time spent in closed arms increased by 31% ($p<0.05$). These behavioral shifts indicated anxiety in rats.

After attachment of D-alanine to C-terminus of Gly-His-Lys molecule, the neurotropic effects of the tripeptide were considerably leveled as in previous modification, and their individual manifestations had the opposite nature. In particular, the number of entries into open arms was reduced after injection of the peptide in doses of 5 μg/kg (by 65%; $p<0.05$) and 50 μg/kg (by 72%; $p<0.05$) as well as the time spent on the central platform after injection of the highest dose (by 42%; $p<0.05$). These behavioral changes, similar to those observed in case of N-terminal localization of D-alanine indicate increased anxiety in rats.

Thus, an anxiolytic effect of Gly-His-Lys peptide was observed after intraperitoneal administration in doses ranging from 0.5 to 50 μg/kg, the lowest dose being most effective. Maximum activity of the peptide used in a low dose typical of regulatory peptides might be achieved via triggering the cascade amplification mechanisms of intracellular formation of a large number of second messenger molecules, function of super-affinity receptors, and existence of acceptor molecules capable of accumulation of circulating signaling molecules [2]. The data on the anxiolytic effects of Gly-His-Lys peptide one more time conform the concept of multifunctional nature of the effects of regulatory peptides [1,5].

TABLE 1. Effect of Gly-His-Lys Peptide on Rat Behavior in EPM ($M\pm m$; $n=10$)

Peptide dose, μg/kg	Time spent in open arms, sec	Time spent in closed arms, sec	Number of entries into open arms	Number of entries into closed arms	Time spent on central platform, sec
Control	30.4±7.9	217.1±18.7	1.2±0.5	5.1±0.8	52.3±13.5
0.5	71.6±9.0*	132.3±16.5*	3.7±0.5*	5.4±0.7	109.9±22.8*
5	64.9±8.8*	138.5±11.9*	3.7±0.5*	6.8±0.7	96.6±12.1*
50	39.6±7.5^{+o}	169.3±11.3^{*+}	2.6±0.5*	7.4±0.7^{*+}	91.1±8.3*

Note. *$p<0.05$-0.01 in comparison with the control; $p<0.05$ in comparison with $^{+}$0.5 μg/kg, ^{o}5 μg/kg.