Effect of Glyprolines on Homeostasis of Gastric Mucosa in Rats with Stress Ulcers

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Pro-Gly-Pro and its metabolite Gly-Pro effectively prevented the development of erosive and ulcerative lesions of the gastric mucosa in rats under conditions of water-immersion restraint stress by restoring the oxidatant-antioxidant balance in the total fraction of gastric mucosa cells. Pro-Gly was least effective in this respect. We conclude that glyprolines hold much promise as pharmaceutical products, which can be used in gastroenterological practice for the prevention and therapy of ulcer disease of the stomach and duodenum.

Key Words: *glyprolines; stress; gastric mucosa; lipid peroxidation; antioxidant defense enzymes*

Ulcer disease of the stomach and duodenum is a serious and unsolved medical problem. According to world statistics, ulcer disease is diagnosed in 10% European people [6]. Conservative therapy is followed by healing of gastroduodenal ulcers in 80% patients. However, disease relapses are observed in 60% patients [5]. Therefore, the search for new prophylactic and therapeutic drugs is an urgent problem. Short peptides hold much promise in this respect. They contain glycine and proline and belong to a family of regulatory peptides (glyprolines) [1,2]. This family consists of Pro-Gly-Pro (PGP), Gly-Pro (GP), Pro-Gly (PG), and other peptides. Glyprolines are endogenous regulatory peptides. They are formed during the synthesis and degradation of collagen, elastin, and othe connective tissue proteins [2]. Many glyprolines demonstrate protective and therapeutic properties on various models of gastric ulceration (ethanol-induced ulcers, stress ulcers, indomethacin-induced ulcers, and ulcers due to pylorus ligation or administration of substance 48/80) [1,3,4]. Previous studies showed that ulcer disease of the stomach and duodenum is accompanied by changes in lipid peroxidation (LPO). These processes are followed by accumulation of peroxidation products and inhibition of the antioxidant defense (AOD) system [6]. *In vitro* experiments showed that tripeptide PGP significantly reduces death of cultured cells during oxidative stress [7]. These data suggest that glyprolines exhibit antioxidant activity and can be used in medical practice.

Here we studied the effects of PGP and its metabolites (GP and PG) on LPO and enzyme activity of ADS in the gastric mucosa (GM) of rats with experimental ulcers.

MATERIALS AND METHODS

Acute experiments were performed on 50 male Wistar rats weighing 200-220 g. The animals were deprived of food, but had free access to water for 1 day before the experiment. Ulcerative and erosive lesions of GM were produced by water-immersion restraint stress [10]. The animals were immobilized in perforated metal tubes with transparent perforated Plexiglas windows on the ends of these tubes. The tubes were vertically

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immersed in a bath with water at 22-23°C for 3 h (up to the level of animal's neck).

The animals were divided into 5 groups (10 rats per group). Group 1 animals (intact control) received intraperitoneal injection of placebo (0.4 ml physiological saline). Group 2 animals (stressed control) received intraperitoneal injection of 0.4 ml physiological saline (placebo) 15 min before immobilization. Group 3, 4, and 5 animals received an intraperitoneal injection of PGP, GP, and PG (3.7 μmol/kg in 0.4 ml physiological saline), respectively, 15 min before immobilization. Peptides PGP, GP, and PG were synthesized in the Laboratory of Regulatory Peptides (Institute of Molecular Genetics, Russian Academy of Sciences). The animals were killed by cervical dislocation. The stomach was removed, cut along the lesser curvature, turned inside-out (mucosa out), and thoroughly washed with physiological saline. GM was examined using a gastroscope (transillumination, ×4). The area of ulcers and length of erosions were estimated. The mean area of ulcers and mean length of erosions were calculated for each group of rats.

The content of major LPO products (conjugated dienes) in GM homogenate was measured spectrophotometrically. The content of thiobarbituric acid-reactive (TBA-reactive) substances was estimated from the reaction with TBA. The concentration of Schiff bases was measured fluorometrically. The state of AOD system was evaluated from activities of superoxide dismutase (SOD) and catalase.

The normality of data distribution was verified by Shapiro–Wilcoxon *W* test. At the normal distribution, the samples were compared by Student's *t* test. We found that the data on ulcerative and erosive lesions are not described by normal distribution. Therefore, two independent samples were compared by Mann–Whitney test.

RESULTS

The exposure of rats to water-immersion restraint stress for 3 h was followed by the formation of ulcers (area 11.86±3.43 mm^2) and erosions in GM (length 4.00±2.19 mm). The area of ulcerative lesions was 70% lower in rats receiving PGP 15 min before stress (3.60±1.56 mm^2, $p<0.05$). The area of erosions decreased by 57.5% (up to 1.70±0.59 mm^2), but these changes were statistically insignificant ($p>0.05$).

GP was more potent in preventing the development of stress-induced damage to GM in rats. Administration of this dipeptide was followed by a significant decrease in the area of ulcers (from 16.98±3.32 to 5.69±0.43 mm^2, by 67%; $p<0.05$) and length of erosions (from 2.20±0.47 to 0.56±0.56 mm, by 75%; $p<0.05$). PG had no effect on the area of stress ulcers, but decreased the area of erosions (by 60%; $p<0.05$). We conclude that GP produces the strongest cytoprotective effect. These data are consistent with the results of our previous experiments. PGP and its metabolites prevent the development of gastric injury on another model of stress [4].

The formation of stress ulcers and erosions in GM of rats was accompanied by activation of LPO, which manifested in increased concentration of LPO metabolites in GM homogenate. Table 1 illustrates an increase in the amount of primary products (conjugated dienes, by 58%; $p<0.001$), secondary products (TBA-reactive substances, by 166%; $p<0.001$), and end products of LPO (Schiff bases, by 45%; $p<0.001$). Significant accumulation of secondary products in GM serves as a criterion for primary activation of LPO in cell membranes. The increase in the content of secondary products is typical of persistent oxidative stress. Studying enzyme activity revealed an increase in catalase activity (by 29.5%; $p<0.001$) and tendency to a decrease in SOD activity ($p>0.05$). The relationship between LPO and AOD system indicates that the increase in the content of LPO products is related to excessive formation of prooxidants under conditions of stress-induced ischemia and reoxygenation of the mucosa [5].

The content of primary, secondary, and end products of LPO in GM of rats receiving PGP before

TABLE 1. Effect of PGP on the Amount of LPO Products and Activity of AOD Enzymes in Homogenate of Rat GM (*M*±*m*)

Parameter	Control (*n*=10)	Stress (*n*=10)	PGP+stress (*n*=10)
Conjugated dienes, nmol/mg protein	323.38±9.07	511.54±17.89***	364.39±31.10^{+++}
TBA-reactive substances, nmol/mg protein	69.64±3.76	185.13±6.80***	87.60±8.22^{***+++}
Schiff bases, arb. units/mg protein	62.16±2.94	90.21±4.49***	71.58±6.42^{+}
SOD, arb. units/mg protein/min	0.19±0.01	0.17±0.01	0.15±0.01*
Catalase, μmol H_2O_2/mg protein/min	5.39±0.14	6.98±0.26***	5.11±0.40^{+++}

Note. Here and in Table 2: *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to the control; $^{+}p<0.05$ and $^{+++}p<0.001$ compared to stress.

TABLE 2. Effects of GP and PG on the Amount of LPO Products and Activity of AOD Enzymes in Homogenate of Rats GM ($M \pm m$)

Parameter	Control (n=10)	Stress (n=10)	GP+stress (n=10)	PG+stress (n=10)
Conjugated dienes, nmol/mg protein	323.38±9.07	511.54±17.89***	386.88±20.27**+++	509.55±19.13***
TBA-reactive substances, nmol/mg protein	69.64±3.76	185.13±6.80***	79.20±5.81+++	177.81±6.21***
Schiff bases, arb. units/mg protein	62.16±2.94	90.21±4.49***	86.53±6.88**	92.87±8.61***
SOD, arb. units/mg protein/min	0.19±0.01	0.17±0.01	0.15±0.01*	0.17±0.01
Catalase, μmol H_2O_2/mg protein/min	5.39±0.14	6.98±0.26***	6.05±0.40	6.50±0.35**

stress was much lower compared to that in stressed animals of the placebo group. As differentiated from primary and secondary products, the concentration of TBA-reactive substances did not return to the control level (26% higher than in intact control animals; $p<0.05$). Study of the AOD system showed that SOD activity in treated animals was 21.1% lower than in controls ($p<0.001$). These differences were observed even when catalase activity did not differ from the normal level. The inhibition of SOD is probably associated with excessive formation of H_2O_2 (*e.g.*, due to low activity of catalase) [5]. Since after treatment with PGP the content of TBA-reactive substances remains above the control, the decrease in SOD activity is associated with the action of toxic products of peroxidation.

Administration of dipeptide GP before stress exposure was followed by a significant decrease in the concentration of primary, secondary, and end products of LPO in GM (as compared to stressed animals of the placebo group, Table 2). However, the content of conjugated dienes and Schiff bases in these rats was higher than in intact control specimens (by 19.6 and 39.2%, respectively). Changes in activity of AOD enzymes in these animals were similar to that in PGP-treated rats. Catalase activity in rats of the treatment group approached that in intact controls. By contrast, SOD activity in GP-treated rats was 21.1% lower than in the control ($p<0.001$).

The dipeptide GP was least potent in preventing the formation of erosive and ulcerative lesions in GM. Moreover, this dipeptide had an insignificant effect on the LPO-AOD system (no differences between GP-receiving rats and stressed animals of the placebo group). The prevention of stress ulcers by PG is probably mediated by other mechanisms.

The tripeptide PGP and GP (metabolite of PGP) were potent in preventing the development of erosive and ulcerative lesions of GM in rats under stress conditions. Moreover, the oxidant-antioxidant balance of the total fraction of GM cells was shown to return to normal after treatment with these compounds. We conclude that PGP holds much promise as a pharmaceutical product, which can be used in gastroenterological practice for the prevention and therapy of ulcer disease of the stomach and duodenum.

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