Effect of Glyprolines on Norepinephrine Tone of Isolated Rat Aortic Rings

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Glyprolines, short proline-containing peptides (Pro-Gly-Pro, Pro-Gly, and Gly-Pro) in concentrations of 10^{-8}-10^{-4} M decreased norepinephrine tone of the isolated rat aortic rings. The vasodilatory effect of Pro-Gly-Pro and Pro-Gly was associated with NO release from endotheliocytes, while Gly-Pro directly affected vascular smooth muscle cells.

Key Words: *glyprolines; endothelium; blood flow*

Functioning of the gastrointestinal tract depends on coordinated activity of the nervous and humoral systems. Enhancement of aggressive factors and exhaustion of defense components disturb homeostasis in the gastric mucosa (GM), which leads to the development of peptic ulcer disease. Adequate blood flow determines energy-dependent processes (*e.g.*, reparation and migration of cells to damaged regions of the surface epithelium) and maintains homeostasis in GM [14,15]. Reduction of blood flow produced by ethanol, indomethacin, or stress causes damage to gastric mucosa [8,13]. The incidence of peptic ulcer disease is high. Therefore, the search for new endogenous antiulcer substances that specifically modulate blood flow in GM is an urgent problem.

Short proline-containing peptides glyprolines (Pro-Gly-Pro, Pro-Gly, and Gly-Pro) possess protective and therapeutic antiulcer properties [1,2,4,12]. Tripeptide Pro-Gly-Pro does not modulate the basal blood flow in the stomach. However, this substance *in vivo* stimulates blood flow reduced after treatment with indomethacin and ethanol [6]. This action can be realized not only via the central mechanisms, but also via the direct effect of glyprolines on the vascular tone. Here we studied these effects of glyprolines.

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MATERIALS AND METHODS

Experiments were performed on ring segments of the left aortic arch from rats weighing 200-220 g. We used the standard method of Blattner and Klassen. Each experiment was performed on one animal. The isolated preparation was maintained in an 8-ml thermostated flow chamber (37°C, 5% CO_2 and 95% O_2, 5 ml/min). Complete Krebs—Henseleit solution served as physiological saline (pH 7.3).

Contractions were recorded on a Harward Apparatus Isometric Muscle transducer using a Hitachi 056 automatic recorder. The initial load was 1 g. The duration of relaxation was 40 min. After relaxation the preparation was treated with norepinephrine in a concentration of 10^{-7} g/ml (Serva). Norepinephrine-induced constrictions were equal to 0.25±0.01 g (n=35). During continuous treatment with norepinephrine this constriction persisted for more than 3 h. In each experiment the value of norepinephrine tone was taken as 100%. The reactions produced by the test substances were compared with the norepinephrine-induced response. The integrity of the endothelium was determined by treatment with acetylcholine in a concentration of 10^{-6} g/ml (Serva). Acetylcholine caused dilation equal to 37.13±6.93% (n=33), which indicated that the endothelium remained intact. Vasomotor properties of Pro-Gly-Pro, Pro-Gly, and Gly-Pro in concentrations of 10^{-12}-10^{-4} M were studied after recovery of the initial norepinephrine tone. The test sub-

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stances in increasing concentrations were added to the bathing solution. The effects of peptides developed immediately after administration. Peptide-produced changes were maximum 3-4 min after treatment (typical experiment, Fig. 1). Mechanical denudation of the vascular segment was performed to evaluate the role of the endothelium in vasodilatory effects of glyprolines. Completeness of this procedure was determined by the absence of reactions to acetylcholine. Apart from mechanical denudation, we used nonselective nitric oxide (NO) synthase blocker N-nitro-L-arginine methyl ester (L-NAME, Sigma). The test peptides were synthesized at the Institute of Molecular Genetics.

The results were analyzed by ANOVA (Statistica software).

RESULTS

Glyprolines dose-dependently reduced norepinephrine tone, which attested to their vasodilatory activity.

The test glyprolines produced minimum vasodilatory effect in a concentration of 10^{-8} M. Pro-Gly-Pro (n=10), Pro-Gly (n=9), and Gly-Pro (n=9) in the maximum concentration (10^{-4} M) caused maximum response (39.9±3.8, 33.5±1.9, and 50.2±6.8%, respectively, Fig. 2).

The majority of humoral regulatory effects on the vascular tone are realized via endotheliocytes possessing high secretory and receptor activity. The integrity of the endothelial lining plays an important role in this process [7,11]. Experiments with denuded preparations were performed to evaluate the role of the endothelium in the vasodilatory effect of glyprolines.

Denudation completely abolished the effect of acetylcholine (control for complete denudation) and peptides Pro-Gly-Pro (n=5) and Pro-Gly (n=5). These findings suggest that endothelium plays a role in the vasodilatory effect of these peptides. As differentiated from Pro-Gly-Pro and Pro-Gly, denudation did not modulate the effect of Gly-Pro in concentrations of 10^{-8}-10^{-4} M (n=6, Fig. 3). Before and after denudation Gly-Pro in a concentration of 10^{-4} M produced maximum reactions (50.2 and 43.5%, respectively).

Our results indicate that the effects of Pro-Gly-Pro and Pro-Gly are mediated by endotheliocytes. Probably, these peptides bind to specific receptors on endotheliocytes or directly affect the membrane of these cells and modulate its properties.

Vasodilatory activity of Gly-Pro was not associated with the influence on endotheliocytes. Probably, this peptide produces a direct effect on vascular smooth muscle cells. It should be emphasized that norepinephrine tone of the denuded vascular segment returned to normal after treatment with Gly-Pro in a concentration of 10^{-6} M, but not in concentrations of 10^{-5} and 10^{-4} M. It can be hypothesized that this dipeptide in high concentrations triggers irreversible intracellular reactions.

The effects of most endogenous vasodilators are related to NO release from endotheliocytes [7]. In the next series Pro-Gly-Pro (n=4) and Pro-Gly (n=4) were applied against the background of NO synthase blockade with L-NAME. The reactions to Pro-Gly-Pro, Pro-Gly, and acetylcholine were abolished after 10-min perfusion of test peptides with L-NAME in a concentration of 3×10^{-6} M. These data suggest that NO serves as a secondary messenger for the vasodilatory action of Pro-Gly-Pro and Pro-Gly.

Our results suggest that the vasodilatory effects of glyprolines Pro-Gly-Pro, Pro-Gly, and Gly-Pro are mediated by various mechanisms. Peptides Pro-Gly-Pro and Pro-Gly modulate NO release from endothe-

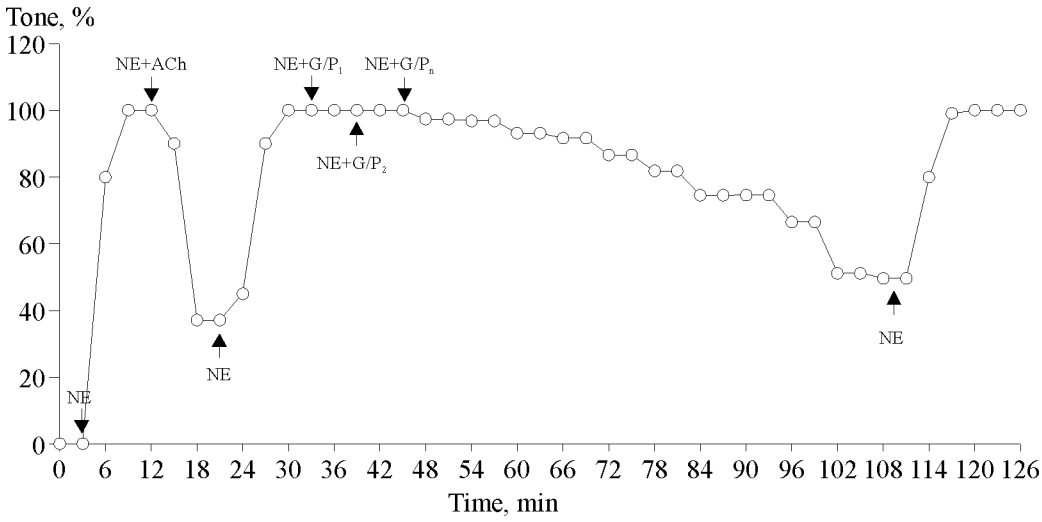

Fig. 1. Typical scheme of the experiment. Ordinate: tone of the isolated preparation after treatment with norepinephrine (NE, 10^{-7} g/ml), acetylcholine (ACh, 10^{-6} g/ml), and glyproline in various concentrations (G/P_1, G/P_2, and G/P_n). Arrows: infusion of the substance.

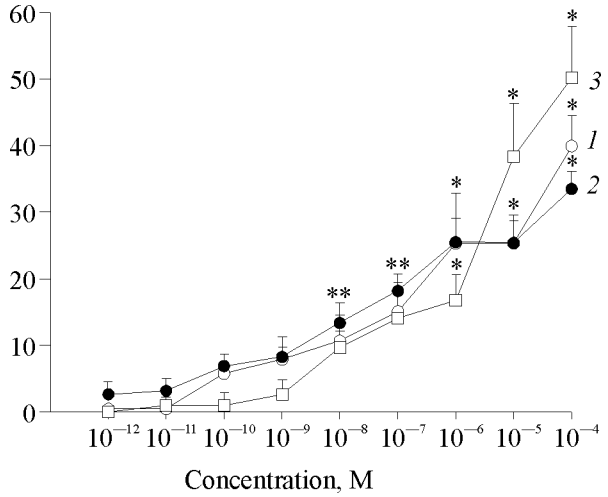

Fig. 2. Vasodilatory effects of glyprolines in increasing concentrations. Here and in Fig. 3: ordinate: vasodilation in % of maximum norepinephrine tone. *p<0.0001 and **p<0.01 compared to norepinephrine.

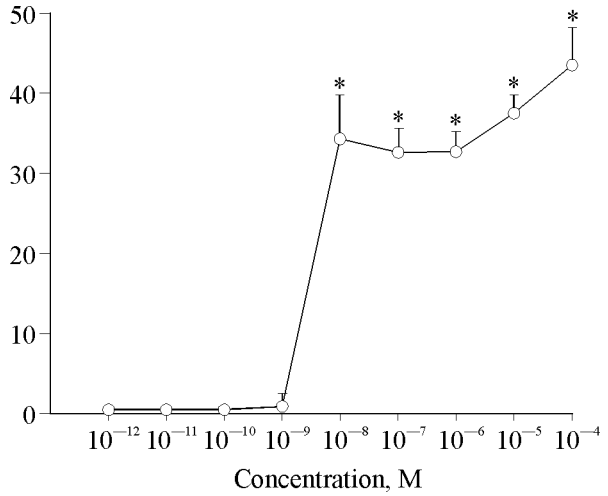

Fig. 3. Vasodilatory effects of Gly-Pro on denuded vascular preparation.

liocytes, while Gly-Pro directly acts on vascular smooth muscle cells. Vasodilatory activity of Pro-Gly and Gly-Pro is realized via different mechanisms, which is consistent with various effects of these dipeptides on the blood flow in the stomach reduced by ulcerogenic factors. Pro-Gly and Gly-Pro *in vivo* slightly decrease basal blood flow. However, these dipeptides produce various effects on blood flow reduced after treatment with indomethacin. Under these conditions Pro-Gly stimulates blood flow, while Gly-Pro is ineffective [6]. The antiulcer effect of Gly-Pro differs from that of Pro-Gly. Antiulcer activity of Gly-Pro and Pro-Gly is manifested when gastric mucosal injuries are mediated by central and peripheral mechanisms, respectively. As differentiated from dipeptides, Pro-Gly-Pro possesses antiulcer properties during ulceration produced both by stress (central mechanism) and treatment with ethanol (peripheral mechanisms) [1-3,5].

Stress produces considerable changes in functional activity of the adrenergic sympathetic system. Its mediators reduce blood flow via constriction of arterioles [10]. Probably, Gly-Pro possessing dilating activity attenuates or blocks vasoconstriction during stress, normalizes blood flow, and reduces the severity of stress-induced damage to GM. These properties can contribute to the antiulcer effect of Gly-Pro under stress conditions. It cannot be excluded that glycoproteins affect not only vascular tone, but also other physiological parameters (*e.g.*, rheological properties of the blood). Repeated intravenous or intranasal treatment with the tripeptide Pro-Gly-Pro accelerates fibrinolysis, increases tissue plasminogen level, and decreases the concentration of plasmin inhibitors [4,9].

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