INTRODUCTION

There were three “stones” of background for the present investigation: the knowledge about adaptive cytoprotection, ischemic preconditioning and gastroprotection by glucocorticoids released in response to stress.

Thirty years ago, in 1979, Andre Robert showed that exogenous prostaglandins (PG), by a mechanism other than the inhibition of gastric acid secretion, maintain the cellular integrity of the gastric mucosa and due to this prevent “gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl and thermal injury”. This property of prostaglandins is called “cytoprotection” (1). Four years later, in addition to a phenomenon of “cytoprotection” (or direct cytoprotection) caused by exogenous PGs, Andre Robert described a phenomenon of “adaptive cytoprotection”, the protection elicited by mild irritants against the gastric injury produced by necrotizing agents (2). He demonstrated that endogenous PG released in response to mild irritants provide this phenomenon and proposed that the increased synthesis of PG may represent a physiological defense mechanism that is important to maintain cellular integrity of the gastrointestinal mucosa (2). This perspicacious proposition has been confirmed and further developed by multiple investigations. It was demonstrated that other factors such as nitric oxide (NO) and sensory nerves participated in realization of gastric protective effect of ischemic preconditioning in cooperation with PG (3-5). The adaptive cytoprotection is considered now as one of the important forms of the gastroprotection in animals and in human (6).

Phenomenon of adaptive cytoprotection was reproduced in various experimental models including stress conditions (5, 7). Recently researchers’ interest has been attracted to the model of ischemic preconditioning in the stomach which is considered as a model of adaptive cytoprotection (6, 8). Attenuation of gastric damage caused by prolonged ischemia-reperfusion by earlier brief ischemia-reperfusion is defined as the gastric ischemic preconditioning (8). It was shown that PG derived from cyclooxygenase-1 and cyclooxygenase-2 as well as NO and sensory nerves are involved in mechanism provided gastroprotective effect of ischemic preconditioning probably by causing vasodilatation and enhancement of the gastric blood flow (6).

Mild stressors are known to induce an increase in glucocorticoid productions. It would be reasonable to assume that these hormones are involved in realization of gastroprotective...
effect of mild stress on the gastric mucosa. However, this possibility was not assumed previously, apparently due to the prevailing traditional point of view on ulcerogenic action of glucocorticoids released during stress. Our previous findings (9, 10) support the idea that glucocorticoids released during stress-induced activation of the hypothalamic-pituitary-adrenocortical (HPA) axis act as gastroprotective hormones but not as ulcerogenic agents as has been generally accepted for several decades. It has allowed us to hypothesize for the first time that glucocorticoids contribute to gastroprotective effect of preconditioning stress. Our recent results confirm that glucocorticoids released during preconditioning mild stress indeed contribute to the protective effect of this stress on gastric mucosa against cold-restraint-induced gastric lesions (7). Accordingly our previous data the maintenance of gastric blood flow is one of a pivotal mechanism of gastroprotective action of glucocorticoids (11, 12). The beneficial action of glucocorticoids on the gastric blood flow is especially important during inhibition of PG or desensitization of capsaicin-sensitive afferents (12, 13). The results mentioned above allow suppose that glucocorticoids contribute to gastroprotective effect of ischemic preconditioning. The present study was designed to verify this supposition.

MATERIAL AND METHODS

Animals

Adult male Sprague Dawley rats (Stolbovoe, Moscow, Russia), weighing 250-300 g were used. Five animals were housed per cage, and animals were acclimatized to standard laboratory conditions (12:12-h light-dark cycle, temperature 20±1°C, free access to food and water) for 7 days before use. The animals were kept in cages with raised mesh bottoms to prevent coprophagy and deprived of food but allowed free access to tap water for 24 h before the experiment. The experiments were performed according to the Helsinki agreement on the guiding principles for research involving animals and human beings. The experiments were approved in the institutional scientific council.

Induction of gastric injury by ischemia-reperfusion and ischemic preconditioning

Ischemia-reperfusion gastric injury was produced accordingly to the method of Wada et al. (14). Under an anesthetic the abdomen was opened, the celiac artery clamped with a small vascular clip for 30 min and then reperfusion was established by removal of the clip. Gastric erosions developed in corpus mucosa were estimated in 3 h after the onset of reperfusion. Rats were exposed to this prolonged ischemic gastric-reperfusion (30 min clamping of celiac artery followed by 3 h of reperfusion, in total 3.5 h) alone or with brief preliminary ischemic-reperfusion preconditioning (two 5 min episodes of clamping of celiac artery followed by 10 min reperfusion, in total 30 min). The schedule of the ischemic-reperfusion preconditioning was selected on the base of the data of literature (8). The control rats were subjected to the same surgical procedure as the respective experimental group, but the celiac artery was not occluded. All animals were anesthetized throughout experiment. Rometar (©SPOFA, Luberec, Czech Republic, 10 mg/kg, i.p.) and Zoletil (©Virbac, Carros, France, 7.5 mg/kg, i.p.) were given in a volume of 0.5 ml/kg body weight for anesthesia. At the end of the 3 h reperfusion, the anesthetized animals were killed by decapitation and stomachs were removed for measuring the areas of erosion. The area (in mm2) of hemorrhagic lesions developed in the corpus mucosa was measured using computer program Image J, summed per stomach, and used as a lesion score.

Methodical approach

To verify whether glucocorticoids contribute to gastroprotective effect of ischemic preconditioning we compared the effects of brief ischemic-reperfusion preconditioning on gastric injury caused prolonged ischemia-reperfusion in rats with normal and deficient corticosterone production as well as in rats with normal and occupied glucocorticoid receptors. Glucocorticoid deficiency was created by adrenalectomy or inhibition of glucocorticoid synthesis by metyrapone. The antagonist of glucocorticoid receptors RU-38486 was used for occupation of glucocorticoid receptors.

The experiments were carried out: 1) in adrenalectomized rats without or with corticosterone replacement and in sham-operated animals; 2) in rats pretreated by metyrapone and in control animals; 3) in rats pretreated by glucocorticoid receptors antagonist RU-38486 and in control animals.

Adrenalectomy was performed one week before experiment under ether anesthesia. Sham-operated rats were subjected to the same surgical procedure, but the adrenals were not removed. After surgery adrenalectomized rats were provided with a 0.9% NaCl solution in addition to tap water in the home cage. Corticosterone replacement was performed by injecting corticosterone (Sigma, Steinheim, Germany, 4 mg/kg in 1 ml/kg 1,2-propylene glycol s.c.) to adrenalectomized rats 15 min before the onset of brief ischemia-reperfusion. The rats without corticosterone replacement were injected with the same volume of vehicle at the same time.

To inhibit glucocorticoid synthesis throughout brief ischemia-reperfusion, metyrapone (Sigma, Steinheim, Germany, 30 mg/kg/ in 5 ml/kg NaCl with drop of Tween-80, i.p.) was injected 20 min before the onset of short ischemia-reperfusion or 50 min before prolonged ischemia-reperfusion (in the case of experiments without ischemic preconditioning). Control rats were received vehicle instead of metyrapone.

The antagonist of glucocorticoid receptors RU-38486 (Sigma, Steinheim, Germany, 20 mg/kg, in 5 ml/kg 1,2-propylene glycol, i.p.) were injected 2 h before the onset of ischemic preconditioning or 2.5 h before the onset of prolonged ischemia-reperfusion (in the case of experiments without ischemic preconditioning). Control animals were injected with respective vehicle.

Estimation of blood corticosterone levels

The trunk blood for measurement of corticosterone levels was collected after decapitation of rats after prolonged ischemia-reperfusion. In some experiments corticosterone levels were additionally tested after short ischemia-reperfusion (ischemic preconditioning). The blood samples were centrifuged at 4°C, and the plasma was frozen for hormonal analysis. Corticosterone level of plasma was measured by microfluorometry. Intra- and interassay variation of measurements was 5.1% and 7.4%, respectively.

Statistical analysis

Data are shown as the mean±SE. We used the nonparametric Mann-Whitney test for comparing erosion scores and Student’s- t test to analyze corticosterone data. In each case, the required level for significance was considered to be P<0.05.

RESULTS

Prolonged ischemic-reperfusion caused gastric lesions in the glandular stomach, with a lesion score between 10 and 15 mm2 in the various controls groups (Figs. 1-5). In control rats the brief...
ischemia-reperfusion (ischemic preconditioning) induced plasma corticosterone rise observed 30 min after the onset of the preconditioning and significantly attenuated gastric lesions caused by prolonged ischemia–reperfusion (Fig. 1).

It is important to emphasize that ischemic preconditioning induced an acute corticosterone rise: significant differences in corticosterone levels between rats without or with ischemic preconditioning were observed 30 min after the onset of the preconditioning (Fig. 1, 3A). After prolonged ischemia-reperfusion there were no significant differences in corticosterone levels between rats without or with preliminary ischemic preconditioning (Fig. 2, 3B, 5).

Fig. 2 demonstrates the effects of ischemic preconditioning on corticosterone levels and gastric lesions observed after prolonged ischemia–reperfusion in adrenalectomized rats without or with corticosterone replacement and in control, sham-operated, animals. In sham-operated animals the preliminary brief ischemia-reperfusion significantly decreased the average area of gastric lesions caused by prolonged ischemia-reperfusion. Although ischemic preconditioning induced an acute corticosterone rise in sham-operated rats, after prolonged ischemia-reperfusion we could distinguish anything in the corticosterone levels between sham-operated animals without or with ischemic preconditioning. Adrenalectomy created a deficiency in corticosterone production: corticosterone levels observed in adrenalectomized rats after prolonged ischemia-reperfusion were very low independently whether or not the animals were underwent ischemic preconditioning. Adrenalectomy by itself significantly aggravated the gastric ulceration produced by prolonged ischemia-reperfusion. In adrenalectomized rats the gastroprotective effect of ischemic preconditioning was prevented: there were no significant differences in the average area of gastric lesions caused by prolonged ischemia-reperfusion between adrenalectomized animals without or with ischemic preconditioning. An acute corticosterone replacement (4 mg/kg, s.c.) mimicking the corticosterone rise in adrenalectomized rats protected the gastric mucosa of these animals against ulcerogenic action of prolonged ischemia-reperfusion. The hormonal pretreatment effect looked like the gastroprotective effect of ischemic preconditioning in sham-operated animals (Fig. 2).

Comparison of the results on corticosterone levels obtained in control groups in various time intervals and presented in Fig. 3A and Fig. 3B demonstrate that ischemic preconditioning induced only an acute corticosterone rise as we mentioned above. Metyrapone pretreatment prevented corticosterone rise, caused by ischemic preconditioning and, moreover, resulted in a short-lasting inhibition of corticosterone production (Fig. 3A). In both metyrapone-pretreated groups, with ischemic preconditioning and in respective group without the preconditioning, corticosterone level (observed in 30 min after the onset the brief ischemia-reperfusion or respective control manipulations) was even smaller than that in vehicle-pretreated group without the preconditioning. After prolonged ischemia-reperfusion we did not observed differences in corticosterone levels between control and metyrapone-treated groups (in both cases: with or without the preconditioning) (Fig. 3B). Metyrapone pretreatment by itself (without ischemic preconditioning) had no influences on gastric lesions caused by prolonged ischemia-reperfusion (Fig. 4). However, metyrapone pretreatment prevented the protective action of ischemic preconditioning was prevented: there were no significant differences in the average area of gastric lesions caused by prolonged ischemia-reperfusion between adrenalectomized animals without or with ischemic preconditioning. An acute corticosterone replacement (4 mg/kg, s.c.) mimicking the corticosterone rise in adrenalectomized rats protected the gastric mucosa of these animals against ulcerogenic action of prolonged ischemia-reperfusion. The hormonal pretreatment effect looked like the gastroprotective effect of ischemic preconditioning in sham-operated animals (Fig. 2).

**Fig. 1.** Effect of brief ischemic-reperfusion preconditioning (I/R-P) on corticosterone levels observed 30 min after the onset of I/R-P and gastric lesions caused by prolonged (3.5 h) ischemia-reperfusion. Data are presented as mean±SE from 8-13 rats/group. Significant difference at P<0.05; * from Control (without I/R-P).

**Fig. 2.** Effect of I/R-P on corticosterone levels and gastric lesions caused by prolonged ischemia-reperfusion in sham-operated (Sham) and adrenalectomized (ADX) rats without or with corticosterone replacement (ADX+Cort). Data are presented as mean±SE from 8-16 rats/group. Significant difference at P<0.05; * from Sham and ADX+Cort; # from Sham without I/R-P and from ADX.
preconditioning on gastric ulceration produced prolonged ischemia-reperfusion. Moreover, in metyrapone-pretreated rats ischemic preconditioning resulted in an aggravation of gastric lesions caused by prolonged ischemia-reperfusion instead of its attenuation. The average area of gastric lesions caused by prolonged ischemia-reperfusion in metyrapone-pretreated animals with ischemic preconditioning was significantly larger comparing that in all other groups (Fig. 4).

The administration of glucocorticoid receptors antagonist RU-38486 before the brief ischemic-reperfusion resulted in an increase of corticosterone levels observed after prolonged ischemia-reperfusion (Fig. 5). The corticosterone increase after RU-38486 pretreatment is a good marker of glucocorticoid receptor occupation. Similar increases were found in both RU-38486-pretreated groups: with or without ischemic preconditioning (Fig. 5). RU-38486 administration by itself significantly aggravated the gastric lesions caused by prolonged ischemia-reperfusion and prevented the protective influence of ischemic preconditioning against gastric injury caused by prolonged ischemia-reperfusion. In RU-38486-pretreated animals there were no differences in the average area of gastric lesions caused by prolonged ischemia-reperfusion between rats without or with ischemic preconditioning (Fig. 5).

**DISCUSSION**

The results obtained show that 30 min gastric ischemia-reperfusion induces an acute plasma corticosterone increase and attenuates gastric lesions caused by 3.5 h ischemia-reperfusion in rats. A decrease of an acute corticosterone rise induced by the ischemic preconditioning or blockade of glucocorticoid receptors prevents the protective effect of ischemic preconditioning on the gastric mucosa. According to these findings, gastric ischemic preconditioning attenuates gastric ischemia-reperfusion-induced injury through involvement of glucocorticoid hormones.

The main task of the study was to verify whether an acute corticosterone rise induced by gastric ischemic preconditioning (which was discovered in the present investigations) participates in a realization of gastroprotective effect of the ischemic

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**Fig. 3.** Effect of metyrapone (30 mg/kg) on corticosterone levels after brief ischemia-reperfusion (A) and prolonged ischemia-reperfusion (B). Data are presented as mean±SE from 10-13 rats/group. Significant difference at P<0.05; * from all groups, # from Controls.

**Fig. 4.** Effect of I/R-P on gastric erosions lesions caused by prolonged ischemia-reperfusion in rats pretreated with metyrapone (30 mg/kg) and control animals. Data are presented as mean±SE from 10-13 rats/group. Significant difference at P<0.05; * from control group without I/R-P, # from all groups.

**Fig. 5.** Effect of I/R-P on corticosterone levels and gastric lesions caused by prolonged ischemia-reperfusion in rats pretreated with RU-38486 (20 mg/kg) and control animals. Data are presented as mean±SE from 10-12 rats/group. Significant difference at P<0.05; * from control group without I/R-P, # from Controls.
preconditioning. To clarify it, we compared the effects of ischemic preconditioning on gastric lesions caused by prolonged ischemia-reperfusion in rats with normal and deficient corticosterone response to the ischemic preconditioning. Solving the task proved to be difficult, due to the complications of inhibiting the corticosterone rise. Two approaches for inhibiting corticosterone rise during the preconditioning were used.

Adrenalectomy with the appropriate corticosterone replacement (4 mg/kg) mimicking an acute corticosterone rise in adrenalectomized rats offered the first approach. Long-lasting glucocorticoid deficiency presented a critical defect of adrenalectomy as a method for our study. However, adrenalectomy in combination with the appropriate corticosterone replacement was a suitable approach for solving the task. In adrenalectomized rats the gastroprotective effect of ischemic preconditioning was prevented but reversed by the acute corticosterone replacement applied immediately before ischemic preconditioning. The corticosterone pretreatment effect in adrenalectomized rats looked like the gastroprotective effect of ischemic preconditioning in control animals with normal corticosterone rise.

Pretreatment by the inhibitor of glucocorticoid synthesis, metyrapone, was the most suitable approach because of a short-lasting inhibiting effect of the drug (15). By metyrapone pretreatment, we were able to prevent the acute corticosterone response and avoid the lasting effects of glucocorticoid deficiency. Metyrapone pretreatment shortly inhibited corticosterone rise, caused by ischemic preconditioning, and prevented the gastroprotective action of ischemic preconditioning. Data with metyrapone pretreatment give strong support for the idea about an involvement of an acute corticosterone rise, induced by ischemic preconditioning, in a realization of gastroprotective effect of the ischemic preconditioning.

Further support for participation of glucocorticoid hormones in the protective effects of ischemic preconditioning on the gastric mucosa comes from the studies using glucocorticoid receptor antagonist RU-38486. In the simultaneous presence of the glucocorticoids and the antagonist in vivo, glucocorticoid receptors are preferentially occupied by the antagonist. The RU-38486-glucocorticoid receptor complex is incapable of nuclear translocation and does not produce a biological effect (9). The protective influence of ischemic preconditioning against gastric injury caused by prolonged ischemia-reperfusion was not observed in the rats pretreated by RU-38486. It is well known that a blockade of glucocorticoid receptors increases corticosterone level probably by activating negative feedback (16). In our experiments RU-38486 pretreatment also stimulated corticosterone production that is a marker of an occupation of glucocorticoid receptors by their antagonist.

The protective effects of ischemic preconditioning were first described for the heart. It has been shown that multiple brief ischemic episodes protect the heart from a subsequent sustained ischemic insult (17). The increased tissue tolerance achieved by brief preliminary ischemia has been termed ischemic preconditioning (18). Later this phenomenon has been demonstrated for other organs, including the stomach (19-21). Ischemic preconditioning is considered as a powerful intervention that glucocorticoid hormones contribute to realization of a phenomenon of “adaptive cytoprotection”. Ischemic preconditioning participates in a protection of the heart from a subsequent ischemia-reperfusion (6, 28). These facts allowed us propose that glucocorticoids contribute to gastroprotective effect of ischemic preconditioning. This proposition was also supported by data of literature. It is known that glucocorticoids, being antioxidants, protect tissues from ischemia (29). Ischemic preconditioning prevents the increase in adhesion of leukocytes to endothelial cells and glucocorticoids are capable to reduce adhesion of leukocytes to endothelial cells (30).

The present study is a consecutive development of our previous investigations about gastroprotective role of glucocorticoids. We demonstrated that a contribution of glucocorticoid hormones to gastroprotection involves their beneficial influences on gastric microcirculation (11-13). In turn, prevention or reduction of microcirculation disturbances is considered as one of the main mechanisms of ischemic preconditioning (6, 28). These findings allowed us propose that glucocorticoids contribute to gastroprotective effect of ischemic preconditioning. This proposition was also supported by data of literature. It is known that glucocorticoids, being antioxidants, protect tissues from ischemia (29). Ischemic preconditioning prevents the increase in adhesion of leukocytes to endothelial cells and glucocorticoids are capable to reduce adhesion of leukocytes to endothelial cells (30).

The results of the present study further develop our idea about important gastroprotective role of glucocorticoids during action of various ulcerogenic factors (9, 10). The present findings demonstrate for the first time that glucocorticoids contribute to protection of the gastric mucosa against the lesions caused by prolonged gastric ischemia-reperfusion. Indeed, adrenalectomy (induced a lasting glucocorticoid deficiency) or RU-38486 pretreatment (induced a lasting blockade of glucocorticoid receptors) significantly aggravated gastric lesions produced by 3.5 h ischemia-reperfusion. In the same time, metyrapone pretreatment (induced only a short-lasting glucocorticoid deficiency) influenced neither plasma corticosterone levels nor a severity of gastric lesions caused by 3.5 h ischemia-reperfusion.

The present experiments were mainly designed to test a participation of glucocorticoid hormones in the protective effect of ischemic preconditioning in the gastric mucosa. The three approaches taken together argue for an involvement of glucocorticoids in realization of the gastroprotective action of ischemic preconditioning.

The data obtained are in agreement with our previous findings demonstrating that glucocorticoids released during preconditioning mild stress contribute to the protective effect of this stress on gastric mucosa against cold-restraint stress-induced gastric injury (7). Taken together, the results suggest that glucocorticoid hormones contribute to a realization of a phenomenon of “adaptive cytoprotection”.

In summary, the present results demonstrate for the first time that glucocorticoid hormones released in response to ischemic preconditioning participate in protective action of ischemic preconditioning on the gastric mucosa against injury caused by prolonged ischemia-reperfusion.


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