INTRODUCTION

Physical activity is crucial for maintaining health. Sedentary lifestyle increases risk for cardiovascular and metabolic diseases (1, 2). Physical exercise can exert both beneficial and harmful effects on the gastrointestinal tract (3-5). As rule, negative effects of physical activity are associated with high-intensity exercise. Peptic ulcers and other gastrointestinal pathological symptoms are common in athletes, especially in marathon runners (6-9). High prevalence of ulceration has been also showed in racehorses (10) and sled dogs (11, 12). At the same time moderate physical activity decreases a risk of gastrointestinal injury (5, 13, 14). To understand how the gastrointestinal injury depends on the intensity and duration of physical activity further animal experiments are needed.

In athletes high-intensity physical activity is often associated with using of non-steroidal anti-inflammatory drugs (NSAIDs) including indomethacin to reduce pain and inflammation caused by training or injury (15). NSAIDs exert their beneficial analgesic and anti-inflammatory action by inhibiting the activity of cyclooxygenases, a family of enzymes that are involved in synthesis of prostaglandins. However deficiency of house-keeping prostaglandins caused by NSAIDs leads to side effects: negative events in the gastrointestinal tract, cardiovascular system and other organs (16-18). The ability of NSAIDs to cause hemorrhagic erosions and ulceration in the gastrointestinal tract is well documented in humans (18, 19) and animals (16, 20, 21). The data obtained in athletes confirm it: NSAIDs consumed by marathon runners increased the incidence of gastrointestinal (bleeding), renal and cardiovascular adverse events (22). Thus, NSAIDs-induced side effects are life-threatening events and the question how to reduce these pathological consequences is of particular concern to clinicians.

Stress preconditioning may increase the resilience of gastric mucosa to ulcerogenic stimuli through the mechanism associated with glucocorticoids released in response to stressor (23). Previously we also found the protective effect of stress preconditioning against ulcerogenic action of indomethacin (IM) (24). Physical activity is a natural stressor activating the hypothalamus-pituitary-adrenocortical (HPA) axis (25-27). However, effects of physical activity as stress preconditioning on the vulnerability of gastric mucosa to ulcerogenic stimuli are not studied yet.

Forced treadmill running and voluntary wheel running are common used as models of physical activity in animal experiments (28-30). However, very little is known on the effects of forced and voluntary running on the gastrointestinal tract. It is known that forced treadmill running before colitis induction in rodent resulted in both the attenuation (31) and exacerbation of colonic damage (32, 33), whereas voluntary...
wheel running only attenuated colon injury (33-35). Additionally, it was shown in the 70s-90s of the last century, that forced as well as voluntary wheel running in the combination of food restriction caused gastric damages in rats (36-39). Thus, more studies are necessary for elucidation of the effects of forced and voluntary running on the gastric injury, especially, on IM or stress-induced gastric injury.

The aim of this work was to study the preconditioning effects of forced (intensive and moderate) treadmill and voluntary wheel running on the vulnerability of the gastric mucosa to ulcerogenic action of IM or cold-restraint stressor. Taking into consideration the gastrotrophic role of glucocorticoids and their contribution to protective effects of stress preconditioning on gastric mucosa (23) we also tested corticosterone levels in the running animals as well as control (sedentary) rats. Since physical activity has significant effect on somatic pain sensitivity (40), we compared tail flick latencies in running animals and sedentary rats under normal conditions (without pathology) as well as under circumstances of gastric injury.

MATERIAL AND METHODS

Animals

Experiments were performed on male Sprague-Dawley rats (Stolbovoe, Moscow, Russia) weighing 270 – 300 g. Six animals per cage 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. They were provided with the laboratory chow and water ad libitum.

The care and treatment of animals were done in accordance with ARRIVE guidelines and EU Directive 2010/63/EU for animal experiments and was approved by the local care committee at the Pavlov Institute of Physiology RAS.

Drugs

We used IM (Sigma, Germany) for induction of gastric erosions. IM (35 mg/kg) suspension was prepared immediately before administration using physiological saline supplemented with drops of Tween 80 (Sigma, Germany) as a vehicle (5 ml/kg).

Forced exercise paradigm

Treadmill running was used as forced exercise paradigm. Treadmill apparatus (Algorithm, Russia) was set up at a 0 incline. The speed of the treadmill was precisely controlled. The animals were separated from each other by opaque partitions. In our experiments two protocols of running were used: “moderate” (9 m/min for 15 min) and “intensive” (15 m/min for 30 min) that were chosen according to the data in the literature that the speed and duration of treadmill running can vary from 10 to 25 m/min and from 15 to 60 min/day, correspondingly (28, 41). Moderate treadmill running was applied in single as well as repeated for 5 days (15 min/day) mode. Intensive treadmill running was used only once. In case when the rats refused to run, they were stimulated with an electric shocks (0.3 mA) delivered at the grids at the rear of the treadmill. The current was determined based on our previous studies and corresponded baseline pain threshold (42).

Moderate running didn’t cause any fatigue or exhaustion in rats. They were active during treadmill session: average sum time of shocks was minimal (4.56 ± 1.1 s for 15 min; n = 23) and the average daily covered distances did not differ significantly, that is why this mode was chosen as repeated for 5 days. However, when the speed was 15 m/min, the sum time of shocks markedly increased (107.8 ± 14.1 s for 15 min; n = 23) indicating the attenuation of activity. The repeated session (next day) did not increase activity. In addition, some of the animals refused to run, that is why intensive treadmill running was used only once.

Before the initiation of the treadmill session, the rats were acclimated to run 5 min at speed of 5 m/min. From 5 min, after warm-up, speed was kept constant. Before and after completion of treadmill running session the animals were kept in their home cage (6 rats per cage). Control (sedentary) animals (6 rats per cage) were housed in their home cages for all time. Treadmill session protocol is created automatically by software installed on computer to work with treadmill apparatus.

Average distance covered by fasting 24 h rats for one intensive treadmill session was 163.1 ± 9.7 m (n = 13). Average distances covered for one moderate treadmill session on day before fasting and on day after 24 h fasting were 115 ± 9 m (n = 10) and 110 ± 5.1 m (n = 10), respectively.

Voluntary exercise paradigm

Wheel running was used as voluntary exercise paradigm. During exercise training animals were placed into individual cages with running wheels (Algorithm, Russia). Rats were given access to running wheels once (2 h/ day) or repeatedly (for 5 days, daily 2 h/day) modes. Average characteristics of voluntary wheel running in fed and fasted animals were given in Table 1. Each wheel was connected to computerized monitoring system to provide a detailed analysis of voluntary wheel running for individual rat. Before and after completion of voluntary wheel running session the animals were in their home cage (6 rats per cage). Control (sedentary) animals were housed in their home cages (6 rats per cage) for all time.

Induction and assessment of gastric injury

Two ulcerogenic stimuli were used to induce the gastric erosions: IM administration and cold restraint stressor. IM was injected into pre-starving (24 h) rats. IM was injected subcutaneously at a single ulcerogenic dose (35 mg/kg in a volume of 5 ml/kg). According to our previous data (43) gastric erosions developed 4 h after a single injection of IM at the dose of 35 mg/kg and healed for 48 h. Based on this data, we examined IM-induced gastric erosions 4 h after IM administration.

To induce gastric injury by cold-restraint stress, pre-starving animals were restrained in special containers and, then, placed into refrigerator under temperature 10°C for 3 h. Lesion areas (mm²) of the stomach was estimated by using the image analysis software (Image J). The area of the gastric injury for each rat included the areas of all the hemorrhagic erosions within glandular part of stomach.

Nociceptive testing

Somatic pain sensitivity was tested by tail flick latency (tail flick test) using a tail-flick meter (Panlab, Harvard Apparatus, Spain). Rats were placed into the container to restrict their movements and then allowed them to habituate for 2 min. Tail flick reflex was induced by the tail’s thermal stimulation, which was discontinued after 10 s to avoid tissue damage (the cutoff point was 10 s). Tail flick latency was measured as the time from the onset of stimulus exposure through tail flick reflex and was automatically recorded in protocol by installed software. For each animal, the tail flick latency was obtained as the mean of three measurements.
Table 1. Average characteristics of repeated voluntary wheel running in rats before and after 24 h fasting.

<table>
<thead>
<tr>
<th>Voluntary running session number</th>
<th>Fasting</th>
<th>Speed (m/s)</th>
<th>Covered distance (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No fasting</td>
<td>0.13 ± 0.08 (17)</td>
<td>196 ± 25 (17)</td>
</tr>
<tr>
<td>2</td>
<td>No fasting</td>
<td>0.12 ± 0.02 (18)</td>
<td>135 ± 33 (18)</td>
</tr>
<tr>
<td>3</td>
<td>Fasting 24 h</td>
<td>0.15 ± 0.01 (18)</td>
<td>185 ± 25 (18)</td>
</tr>
</tbody>
</table>

Table 2. Effects of forced treadmill and voluntary wheel running on indomethacin- and cold restraint-induced gastric erosions.

<table>
<thead>
<tr>
<th>Experiments</th>
<th>Type of voluntary running</th>
<th>Intensity</th>
<th>Type of repetition</th>
<th>Ulcerogenic stimulus</th>
<th>Area of gastric erosions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>Forced treadmill running</td>
<td>Moderate</td>
<td>Single</td>
<td>Indomethacin</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderate</td>
<td>Repeated</td>
<td>Indomethacin</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intensive</td>
<td>Single</td>
<td>Indomethacin</td>
<td>↑</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>Forced treadmill running</td>
<td>Moderate</td>
<td>Single</td>
<td>Cold restraint</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderate</td>
<td>Repeated</td>
<td>Cold restraint</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Intensive</td>
<td>Single</td>
<td>Cold restraint</td>
<td>↑</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>Voluntary wheel running</td>
<td>No data</td>
<td>Single</td>
<td>Indomethacin</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No data</td>
<td>Repeated</td>
<td>Indomethacin</td>
<td>↓</td>
</tr>
<tr>
<td>Experiment 4</td>
<td>Voluntary wheel running</td>
<td>No data</td>
<td>Single</td>
<td>Cold restraint</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No data</td>
<td>Repeated</td>
<td>Cold restraint</td>
<td>↑</td>
</tr>
</tbody>
</table>

† aggravation; ↓ attenuation.

Collection of blood samples and estimation of plasma corticosterone levels

Blood samples were collected from trunk vessels after decapitation. Plasma samples were obtained by blood centrifugation at 3000 revolutions/min, for 15 min at 4°C. Samples were stored at –20°C until further analysis. The concentration of corticosterone in plasma was determined using commercial ELISA kits (K210R, “HEMA”, Russia). Detection level was 5 nmol/l according to the manufacturer.

Experimental design

In all experiments animals were randomly assigned to exercise (voluntary wheel or forced treadmill running) or sedentary (control) groups (6 rats per cage). Sedentary (non-running) rats were housed in their cages throughout period of experiment. The running rats were took out from their cage and placed in treadmill apparatus or individual wheel running cages. After completion of running sessions all rats were put back in their home cage and kept at room temperature.

Experiments were started at 11:00 a.m. The animals were fasted 24 hours before the experiment (Day 0). Next day (Day 1) the rats had a single treadmill or wheel running session and, then, 1 h after completion of running (and keeping in their home cages) they were subjected IM administration or cold restraint stress. In model of repeated running for 5 days the rats were fasted on Day 4, immediately after completion of forced treadmill or wheel running session. On Day 5 they also had treadmill or wheel running session and, then, 1 h after completion of running they were subjected IM injection or cold restraint stress.

In experiment 1 and experiment 2 we studied preconditioning effects of forced treadmill running on IM- and stress-induced gastric injury, respectively (Table 2). Three groups of rats were subjected forced treadmill running: first and second group had a single or regular (daily for 5 days) moderate treadmill session, respectively, while third group had a single intensive treadmill session. Each of running groups had corresponding sedentary control. One hour after completion of single or repeated treadmill session, the running as well as control rats were given IM and, then, decapitated 4 h after its administration (experiment 1) or they were subjected cold restraint stress and decapitated 3 h after the onset of stress (experiment 2).

In experiment 3 and experiment 4 we studied preconditioning effect of voluntary wheel running on IM- or cold restraint-induced gastric injury, respectively (Table 2). Two groups of rats that had single or repeated (for 5 days, 2 h/day) wheel running session were in each of experiments. Each of running groups had corresponding sedentary control. One hour after completion of wheel running session on the Day 1 or Day 5, the both of running and control rats were subjected IM administration (experiment 3) or cold restraint stress (experiment 4) and were decapitated like in experiment 1 and experiment 2.

Somatic pain sensitivity (tail flick latencies) was evaluated under normal conditions and under circumstances of IM-induced gastric injury. Preliminary habituated two groups (running and sedentary) of rats were used for measurement of somatic pain sensitivity. Tail flick latencies were estimated before (baseline tail flick latency) and immediately after completion of forced running or voluntary running sessions and 4 hour after IM administration under IM-induced injury in both of running and sedentary rats.

Data and statistical analysis

Data was expressed as mean ± SEM. Data was analyzed with ANOVA module of the MedCalc Version 12.7.0.0. (Statistics for
biomedical research, MedCalc Software, Belgium). Statistical significances were tested by one or two-way repeated measures ANOVA (factors: group and time), followed by a post hoc Turkey-Kramer test. When Levene's test for homogeneity of variances was significant, nonparametric Kruskall-Wallis test was used. Relationships between plasma corticosterone levels and corresponding areas of gastric erosions were examined using Pearson's correlations. In each case, the required level for significance was considered $p < 0.05$.

RESULTS

Characteristic of forced treadmill and voluntary wheel running as preconditioning stressors

Plasma corticosterone levels induced by single moderate (Fig. 1Aa) or intensive (Fig. 1Ba) running were significantly ($p < 0.05$) higher than in control. Corticosterone rise induced by moderate or intensive treadmill running was accompanied by analgesic effects: a single moderate as well as intensive treadmill running caused an increase in tail flick latencies at once after completion of treadmill running (Fig. 1). Two-way repeated ANOVA was significant for factor group ($F(3,21) = 5.09$) $p < 0.011$; time ($F(1,20) = 20.48$) $p < 0.001$ and their interaction ($F(3,20) = 4.06$) $p < 0.024$). Tail flick latencies induced by moderate or intensive running were also significantly ($p < 0.05$) longer than baseline tail flick latencies (before running) and tail flick latencies in control group (Fig. 1). Similarly, voluntary wheel running in 5 days by itself resulted in an elevation of plasma corticosterone levels ($p < 0.05$) compared to control animals (Fig. 2a). An increase of corticosterone levels was accompanied by analgesic effect (an increase of tail flick latencies, as compared to control animals) (Fig. 2b).

Baseline plasma corticosterone level was $52.11 \pm 4.36$ ng/ml ($n = 12$).

![Fig. 1. Effects of a single moderate (A) and intensive (B) forced treadmill running by itself on plasma corticosterone levels (a) and somatic pain sensitivity (b). Plasma corticosterone levels were measured 15 min (A) or 30 min (B) after the onset of running. Tail flick latencies were measured before (baseline) and immediately after completion of moderate (9 m/min, 15 min) or intensive (15 m/min, 30 min) treadmill session (post-running). Significant differences at $p < 0.05$ * versus control group (a) and all groups (b); $n = 5 – 6$ per group.](image-url)
Fig. 2. Effects of voluntary wheel running by itself on plasma corticosterone levels (a) and somatic pain sensitivity (b). Rats were given access to wheel for 5 days (2 h/day). Tail flick latencies and plasma corticosterone levels were measured immediately after completion of wheel running session (on Day 5). Significant differences at $p < 0.05$ * versus control group; $n = 6 – 12$ per group.

Fig. 3. Preconditioning effects of forced treadmill running on indomethacin (IM)-induced gastric erosions (a) and plasma corticosterone levels (b). IM (35 mg/kg, s.c.) was administered 1 h after the completion of treadmill session: single moderate (9 m/min, 15 min) running (A); repeated moderate running (9 m/min, 15 min/day for 5 days) (B); single intensive running (15 m/min, 30 min) (C). IM-induced gastric erosions and plasma corticosterone levels were measured 4 h after IM administration. Significant differences at $p < 0.05$ * versus control group; $n = 6$ per group.
Effects of forced treadmill running under circumstances of ulcerogenic action of indomethacin

Forced treadmill running by itself did not induced gastric erosions fasted rats.

A single moderate running did not affect (F (1,12) = 0.627, p = 0.447) the vulnerability of gastric mucosa to ulcerogenic IM action (Fig. 3Aa). However, plasma corticosterone levels 4 h after IM administration were significantly lower in running than in control rats (p < 0.05) (Fig. 3Ab).

At the same time repeated moderate running markedly (p < 0.05) decreased IM-induced gastric erosions (Fig. 3Ba). Plasma corticosterone levels 4 h after IM were not significantly different (F (1,12) = 0.457, p = 0.514) in running and control rats (Fig. 3Bb).

A single intensive treadmill running also caused gastroprotective effect (decrease of the mean area of IM-induced gastric erosions) in running rats (Figs. 3Ca and 8A). Gastroprotective effect of treadmill running was accompanied by an increase of plasma corticosterone levels 4 h after IM administration (F (1,31) = 5.547, p = 0.025) (Fig. 3Cb). Baseline plasma corticosterone level was 59.04 ± 15.2 ng/ml (n = 6).

We found differences between tail flick latencies 4 h after IM in running and control rats (Fig. 4). Tail flick latencies 4 h after IM were significantly (p < 0.05) longer in control rats compared to running rats in the both moderate (Fig. 4A) and intensive (Fig. 4B) treadmill groups (F(1,15) = 8.119, p < 0.01; F(1,22) = 4.581, p = 0.04). Tail flick latencies 4 h after IM in intensive running rats were not different from the baseline level (before running) (Fig. 4A) whereas in moderate running rats they were increased (p < 0.02) compared to corresponding baseline latencies (p < 0.01) but were less (p < 0.05) than in sedentary rats (Fig. 4B).

Effects of forced treadmill running under circumstances of ulcerogenic action of cold-restraint

A single moderate running did not affect stress-induced gastric erosions (F (1,24) = 0.013, p = 0.908) (Fig. 5Aa). However, repeated moderate running markedly attenuated stress-induced gastric erosions (F (1,11) = 5.893, p = 0.038) (Fig. 5Ba).

Both single and repeated moderate treadmill running did not influence plasma corticosterone levels 3 h after the onset of cold restraint stress (F (1,24) = 0.577, p = 0.456; (F (1,12) = 0.419, p = 0.532 ) treadmill running (Fig. 5Ab and 5Bb).

A single intensive treadmill running exacerbated stress-induced gastric injury (increased the mean area of gastric erosions (F(1,17) = 5.644, p = 0.03)) (Figs. 5Ca and 8A). Proulcerogenic action of intensive treadmill running on the gastric erosions was accompanied by a reduction stress-induced plasma corticosterone levels (p < 0.05) (Fig. 5Cb).

Baseline plasma corticosterone level was 48.78 ± 14.2 ng/ml (n = 6).

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**Fig. 4.** Somatic pain sensitivity under circumstances of indomethacin (IM)-induced gastric injury in forced treadmill running rats and control rats. Tail flick latencies were measured before running (baseline) and 4 h after IM administration. IM (35 mg/kg, s.c.) was administered 1 h after the completion of treadmill session: repeated moderate running (9 m/min, 15 min/day for 5 days) (A); a single intensive running (15 m/min, 30 min) (B). Significant differences at p < 0.05. *versus corresponding baseline tail flick latencies, †versus control (A) or running (B) group 4 h after IM injection; n = 5 – 6 per group (A); n = 11 – 18 per group (B).
Effects of voluntary wheel running under circumstances of ulcerogenic action of indomethacin

Baseline plasma corticosterone levels measured before wheel running session were 46.7 ± 8.1 ng/ml (n = 12).

A single voluntary wheel running increased the mean area of IM-induced erosions (H(1,24) = 13.25, p = 0.0003) (Fig. 6a). At the same time repeated voluntary wheel running remarkably (p < 0.05) attenuated IM-induced gastric erosions (F(1,11) = 5.817, p = 0.039) (Figs. 6Ba and 8B).

We didn’t find significant differences between IM-induced corticosterone levels in running and control rats after single (p = 0.122) as well as repeated (p = 0.805) voluntary wheel running (Fig. 6Ab and 6Bb).

Effects of voluntary wheel running under circumstances of ulcerogenic action of cold restraint

A single voluntary wheel running increased the mean area of stress-induced erosions (H(1,24) = 4.32, p = 0.037) (Fig. 7a). Similarly, repeated voluntary wheel running also aggravated (H(1,12) = 5.0256, p = 0.025) the gastric erosions caused by cold restraint stress (Figs. 7B and 8B). There were no significant differences between stress-induced erosions in running and control rats after single (p = 0.392) and repeated (p = 0.158) wheel running (Fig. 7Ab and 8B).
corticosterone levels in running and control rats after single (p = 0.539) as well as repeated (p = 0.073) voluntary wheel running (Fig. 7Ab and 7Bb).

**DISCUSSION**

In the present study we found that a single intensive (15 m/min, 30 min) forced treadmill running as well as voluntary wheel running in 5 days exerts gastroprotective effect on IM-induced gastric erosions but pro-ulcerogenic action on cold restraint-induced gastric injury. At the same time regular forced moderate (9 m/min, 15 min) treadmill running in 5 days before ulcerogenic stimulus attenuated both IM- or stress-induced gastric erosions.

Here we used two models of running: forced treadmill running and voluntary wheel running. Forced treadmill running with a fixed speed and duration allowed us to compare the effects of running at different intensities whereas voluntary wheel running was used for evaluation of natural physical activity.

We confirmed here that physical activity stimulates the HPA axis (25-27). Both forced (Fig. 1) and voluntary (Fig. 2) running by itself resulted in an elevation of corticosterone levels suggesting the HPA axis activation. It means that both forced and voluntary running are natural physiological stressors.

Stress-induced analgesia (short-term decrease of pain sensitivity) is one of the signs of the stress response (44). Attenuation of somatic pain sensitivity caused by physical exercise including running is well known in humans and animals (28, 45). In our experiments the HPA axis activation caused by forced or voluntary running was accompanied by an increase of tail flick latencies (decrease of somatic pain sensitivity) at once after completion of running suggesting the development of stress-induced analgesia (Fig. 1 and 2).

We did not discover any differences between effects of moderate and intensive forced running by itself on the HPA axis activity and somatic pain sensitivity in rats. Although plasma ACTH and cortisol concentrations increase linearly with exercise intensity in humans (26, 46), this dependence may be non-linear in animals. The lack of intensity effects on somatic pain sensitivity in our experiments is in line with data of most researchers that development of exercise-induced analgesia under normal conditions is independent on the type of exercise and their intensity (45, 47), suggesting non-specific stressful nature of pain inhibition. Nevertheless, some studies in humans report that greater analgesia developed at high-intensity exercise (48). However, repeated high-intensity exercise can exacerbate pain, and low-intensity exercise attenuate (45).

Based on data of literature we can speculate that moderate and intensive running trigger the different mechanisms (49). Specifically, it has been shown that low-speed treadmill running

**Fig. 6.** Preconditioning effects of voluntary wheel running on indomethacin (IM)-induced gastric erosions (a) and plasma corticosterone levels (b) in rats. Rats were given access to running wheels once (2 h/ day) (A) or repeatedly (for 5 days, daily 2 h/ day) modes (B). IM (35 mg/kg, s.c.) was administered 1 h after the completion of single or repeated voluntary wheel running session. IM-induced gastric erosions and plasma corticosterone levels were measured 4 h after IM administration. Significant differences at p < 0.05 versus control group n = 12 per group (A); n = 6 per group (B).
for 30 min increased c-Fos expression in the dorsal raphe nucleus, whereas high-speed running - in corticotropin releasing factor neurons in the paraventricular nucleus of hypothalamus (49). Low-speed treadmill running-induced effect on neuronal activity was accompanied by a decrease of anxiety and depressive-like behavior (49).

We have showed different effects of single and repeated running on the different types of gastric mucosal lesions (Table 2). Single forced intensive treadmill running exerted gastroprotective effect on IM-induced gastric erosions (Fig. 3) and proulcerogenic effect - on stress-induced gastric injury (Fig. 5). At the same time, a single voluntary running had proulcerogenic action on IM- as well as cold restraint-induced injury (Figs. 6 and 7). Aggravation of the gastric injury induced by running may be due to the combination of severe preconditioning and ulcerogenic stressors of different modality (50-53). In the present study, we consequently (at 1 h interval) applied two stressors of different nature: forced running and cold restraint, combination of which could enhance the ulcerogenic effects of stressor on the gastric mucosa after intensive forced running, but it did not occur with the action of another ulcerogenic stimulus IM. Proulcerogenic effects of a single voluntary running on both type of gastric mucosal lesion may be explained by complex action of many stressors including not only the physical activity, but also strong psychological stressors (novelty, social isolation, fear) that have negative effect on gastric mucosa (54, 55) and may potentiate the action of ulcerogenic stressor on gastric mucosa regardless it nature.

Repeated moderate running in 5 days exerted the gastroprotective effect, regardless the nature of ulcerogenic stimulus (Figs. 3 and 5), which may be due to antidepressant/anxiolytic effects of low-speed treadmill (49). At the same time repeated voluntary running had the protective effect against IM (Fig. 6) and proulcerogenic effect against cold restraint (Fig. 7). According to data of literature the chronic stress can cause the both proulcerogenic (56, 57) and gastroprotective effects (58) on gastric mucosa. Gastroprotective effect of repeated running is in line with our previous data on the development of adaptation under chronic stress (59). However, as we have shown, an increase of severity of ulcerogenic stressor can result in the transformation of gastroprotective effect of chronic stress into its proulcerogenic action (59).

In the present study, the both forced and voluntary running had a gastroprotective effect on IM-induced gastric damage. Gastroprotective effect of intensive treadmill running on IM-induced gastric injury was accompanied by an elevation of IM-induced corticosterone levels 4 h after IM injection (Fig. 3). We suggest that the greater resilience of the gastric mucosa to the ulcerogenic effect of IM, at least, after the intensive running may be due to the gastroprotective action of glucocorticoids released in response to IM action. According to our data glucocorticoids released in response to IM or stress are gastroprotective factors
Their role is especially important under circumstances of deficiency of prostaglandins which play a critical role in the pathogenesis of IM-induced injury (64, 65). Glucocorticoids exert compensatory gastroprotective action under circumstances of ulcerogenic action of IM that may be provided by their beneficial action on links of general homeostasis as well as local protective factors (21, 63, 66-69).

Taken into consideration essential role of glucocorticoids under the IM-induced gastric injury, it may speculate that, corticosterone rise induced by intensive running contribute to gastroprotective effect of IM in addition to protective action of the IM-induced corticosterone rise. On the other hand, corticosterone rise induced by intensive running may inhibit stress-induced corticosterone level through negative feedback.

Fig. 8. Representative photos of stomachs showing gastric mucosal lesion induced by indomethacin (35 mg/kg) (a, b) or cold restraint stress (3 h, 10°C) (c, d) in control and running rats. A - single intensive treadmill; B - repeated voluntary wheel running. Photos demonstrate gastroprotective effects of forced and voluntary running on IM-induced gastric erosions and proulcerogenic effects of forced and voluntary running on stress-induced gastric erosions.
mechanism. In turn, deficiency of glucocorticoids induced by inhibition of the HPA axis activity may attenuate the resilience of gastric mucosa to ulcerogenic stress (60, 61). Indeed, in our experiments proulerogenic effect of intensive treadmill on stress-induced injury was accompanied by a decrease of stress-induced corticosterone levels (3 h after completion of cold restraint) (Fig. 5). We showed the negative (r = -0.5872, p = 0.013) correlation between stress-induced corticosterone levels and areas of gastric erosions under intensive treadmill running indicating lower corticosterone level may contribute to proulerogenic action of cold restraint after intensive treadmill running. It is possible that inhibition of HPA axis activity (through feedback mechanism) developed after elevation of corticosterone levels induced by a single session of voluntary running contributes to its proulerogenic effects on gastric mucosa. However, the verification of the assumption is a task for further studies.

We didn’t find any differences in IM- or stress-induced corticosterone levels between control and running rats when repeated treadmill running (Figs. 3 and 5) or voluntary wheel running in 5 days (Figs. 6 and 7) were used as stress preconditioning. The findings are consistent with our previous data (59) that chronic stress blunted the HPA axis response to acute ulcerogenic stressor. Data of other researchers also suggest that long-term exercise can reduce the HPA axis activity (70-72). However, chronic stress can induce not only habituation, but also hyperresponsiveness to a novel stressor (73). It may be assumed that the different effects of repeated running on IM- and stress-induced gastric erosions may due to different patterns of the HPA axis response to ulcerogenic stressor.

IM-induced gastrointestinal injury is accompanied by adverse events outside the gastrointestinal tract (24, 43, 69, 74), including the changes in somatic pain sensitivity. As we reported earlier, the IM-induced pathological process in the gastrointestinal tract was accompanied by a decrease of somatic pain sensitivity (24, 74, 75). Here, we find that attenuation of IM-induced gastric injury induced by treadmill running was accompanied by normalization of somatic pain sensitivity (Fig. 4). The data obtained is in line with our previous results showing that stress preconditioning has the beneficial influence not only the gastrointestinal tract, but also other physiological parameters: it normalizes somatic pain sensitivity and stabilizes the hemodynamic parameters (24).

Thus, forced intensive as well as voluntary running in regular regimen may have dual effect on the gastric mucosa injury. A regular moderate running protects gastric mucosa against ulcerogenic action of IM and stress. Nevertheless, an increase of intensity of running in the combination with ulcerogenic stressor may be dangerous due to aggravation of stress-induced injury. The data shows the efficiency of regular physical exercises at moderate intensity in protection of gastric mucosa against ulcerogenic stimuli. Physical exercises may be considered as perspective approach for attenuation of adverse effects of NSAIDs therapy on the gastrointestinal tract.

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