INTRODUCTION

Glucocorticoid hormones and gastric ulceration have been discussed in many contexts. Although there is a long-standing debate over whether glucocorticoid therapy leads to peptic ulcer disease in humans, it is established that administration of glucocorticoids to experimental animals can result in an acute gastric erosion formation (1). In the same time, in some cases, administration of glucocorticoids to experimental animals can attenuate gastric erosion formation (2-4). It is also known that basal glucocorticoid production contributes to the maintenance of gastric mucosal integrity. The glucocorticoids may have a permissive role in allowing gastroprotective mechanisms to exert their full potential. A permissive role was suggested in gastric mucosal protection induced by prostaglandins, sulfhydryls, cimetidine (5) or interleukin-1 (6). The most controversial question is the question about the action of glucocorticoids produced during stress. The ulcerogenic properties of exogenous glucocorticoids observed at high pharmacological doses were extrapolated to the properties of endogenous glucocorticoids released in stress situations in large quantities. Consequently, it has been generally accepted for several decades that glucocorticoids released during stress are responsible for ulcerogenic action of stress on the stomach. However, our results do not support the traditional paradigm and suggest that glucocorticoids released during stress-induced acute activation of the hypothalamic-pituitary-adrenocortical (HPA) axis are important gastroprotective factors, allowing us to re-evaluate the traditional view about their ulcerogenic effect (1, 7-10). Therefore, beneficial action of glucocorticoids released in large quantities during acute activation of HPA axis on the gastric mucosa is opposite to the harmful actions of exogenous glucocorticoids used at pharmacological doses.

Thus, in general glucocorticoid hormones may have dual action on the stomach: physiological gastroprotective and pathological proulcerogenic one. In the present study we investigated how gastroprotective action of glucocorticoids can be transformed to proulcerogenic effect. Dose- and time-dependent effects of single injection of dexamethasone on cold-restraint-induced gastric erosions, corticosterone and blood glucose levels, somatic parameters were investigated in fasted rats. Dexamethasone at a dose of 1 mg/kg decreased the gastric erosion area and maintained blood glucose level in fasted rats in the case of its injection 1 h before the stress. A dose of 1 mg/kg has been selected for the time-dependent study. The results demonstrate that single injection of dexamethasone at a dose of 1 mg/kg may attenuate or aggravate cold-restraint-induced gastric erosions depending on the time of the injection before the stress. Gastroprotective action of dexamethasone was observed in the case of its injection 1 and 12 h before cold-restraint. The further increase in the time interval caused transformation of gastroprotective action of dexamethasone to proulcerogenic effect. Both short-term and long-term dexamethasone action resulted in maintenance of blood glucose level in fasted rats. The results suggest that glucocorticoid-induced long-lasting maintenance of blood glucose levels accompanied with the signs of their catabolic effect and dexamethasone-induced corticosterone deficiency may be responsible, at least partly, for the transformation of gastroprotective action of glucocorticoids to their proulcerogenic effect.

Key words: cold-restraint stress, gastric erosions, glucocorticoids, dexamethasone, gastroprotective and proulcerogenic action, transformation, catabolic effects, blood glucose
important for the maintenance of the gastric mucosal integrity (16). On the base of this knowledge we assumed and then demonstrated that the maintenance of glucose homeostasis by glucocorticoid hormones could be a fundamental of their beneficial action on gastric mucosa integrity (8, 11).

Because of maintenance of glucose homeostasis by glucocorticoids could be fundamental of their gastroprotective action (8), it was reasonable to assume that glucocorticoid-induced disturbance of glucose regulation, observed in clinical and experimental situations (13), may contribute to ulcerogenic action of glucocorticoids on the gastric mucosa. We proposed that short-term maintenance of blood glucose level provides the gastroprotective action of glucocorticoids, while long-lasting maintenance of blood glucose level or long-lasting hyperglycemia through a disturbance of carbohydrate regulation may account at least partly for the ulcerogenic action of glucocorticoids. Thus, we hypothesized that glucocorticoid-induced long-lasting maintenance of blood glucose level accompanied by their catabolic effect may be responsible for the transformation of gastroprotective action of glucocorticoids to their ulcerogenic effect. The present study was designed to verify the hypothesis.

MATERIALS 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 24-25 h before the experiment. The experiments were approved in the institutional scientific council.

Methodical approach

To verify whether glucocorticoid-induced long-lasting maintenance of blood glucose level followed by their catabolic effect can be responsible for the transformation of gastroprotective action of glucocorticoids to their proulcerogenic effect, first of all, we developed the experimental model by which the transformation could be demonstrated. With this aim, dose- and time-dependent effects of single injection of dexamethasone on stress-induced gastric erosion formation were investigated. Eventually, we have succeeded in developing the model. Then, stress-induced blood glucose and corticosterone levels as well as the lost of body and thymus, spleen weights (as catabolic markers) were examined in the same experimental conditions in which the transformation of gastroprotective action of dexamethasone to ulcerogenic effect was demonstrated.

Production of gastric erosions and estimation of their severity

Gastric erosions were produced by cold-restraint stress. Rats were restrained individually in immobilization tubes with ventilation holes for 3 h in a cold room (temperature 10°C). At the end of the 3 h stress exposure, the 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.

Experimental protocols

Dexamethasone (Serva, Heidelberg, Germany) was dissolved in 1,2-propylene glycol (Vecton, Shostka, Russia) freshly for each experiment and injected intraperitoneally in a volume of 1 ml/kg. Every dexamethasone-pretreated group had an appropriate control. Control animals received 1 ml/kg of the vehicle at corresponding time.

1. Effects of varying the dose of dexamethasone

In the dose-dependent investigation, the animals were given a single injection of dexamethasone in doses of 0.01, 0.1, 1 and 10 mg/kg 1 h before the onset of cold-restraint. Dexamethasone and its vehicle were administered at 10 o’clock (24 h after the onset of fasting), then, 1 h later, at 11 o’clock, the rats were stressed and decapitated in 3 h.

2. Effects of varying the time of dexamethasone administration

In the time-dependent investigation, we varied the time interval between dexamethasone administration and the stress onset. For this, the animals were given a single intraperitoneal injection of dexamethasone at a dose of 1 mg/kg at various time points: 1, 12, 18, and 24 h as well as 3, 5, and 7 days before the stress. The animals were stressed 24 h after the onset of fasting, and then, in 3 h, decapitated. We fixed the onset of fasting and stress (at 10 o’clock) and varied the injection of dexamethasone.

Estimation of blood corticosterone and glucose levels

The trunk blood for measurement of glucose and corticosterone levels was collected after decapitation of rats 3 h after the onset of stress. A drop of blood was used for measurement of glucose level which was determined with the aid of One Touch Ultra system (LifeScan, Johnson & Johnson company, USA) using the blood glucose sensor electrode. The blood samples for measurement of corticosterone levels were centrifuged at 4°C, and the plasma was frozen for hormonal analysis. Corticosterone level of plasma was measured by microfluorometry (1). Intra- and interassay variation of measurements was 5.1% and 7.4%, respectively.

Estimation of somatic parameters

Body weight was examined first before the onset of fasting and, then, before the onset of stress. We calculated the lost of body weight between these time points (during fasting). In specified cases, in the groups with dexamethasone injection 3, 5, and 7 days before stress the body weight was additionally examined before dexamethasone injection. In these cases we estimated the lost of body weight not only during fasting but also between dexamethasone injection and the onset of fasting.

Thymus and spleen were dissected after decapitation of rats and the weight was measured in pre-weighted tubes.

Statistical analysis

Data are shown as the mean±SE. We used the nonparametric Mann-Whitney test for comparing erosion scores and Student's
The effects of various doses of dexamethasone injected 1 h before cold-restraint on the stress-induced gastric erosions and blood glucose level are presented on Fig. 1. Dexamethasone at a dose of 1 mg/kg significantly reduced the erosion severity and enhanced the blood glucose level observed 3 h after the onset of stress comparing those of control animals. Further 10-fold increase in dexamethasone dose to 10 mg/kg did not cause further potentiation the protective hormonal effect on the gastric mucosa: there were no significant differences in values of mean area of gastric erosions between the groups pretreated by dexamethasone at a dose 1 mg/kg (0.8±0.4 mm²) and 10 mg/kg (0.4±0.2 mm²). A dose of 1 mg/kg has been selected for the time-dependent study.

Fig. 2 demonstrates the effects of varying the time of dexamethasone treatment at a dose of 1 mg/kg relative to the onset of cold-restraint (from 1 h to 24 h) on the stress-induced gastric erosions and blood glucose levels. This figure presents the combined results of several experiments. Each dexamethasone pretreated group has corresponding vehicle control group. Dexamethasone injected at a dose of 1 mg/kg attenuated or aggravated the stress-induced gastric erosions depending on the time of its injection (Fig. 2). A reduction in gastric erosion area was observed when dexamethasone was injected at 1 and 12 h before the onset of stress. Dexamethasone did not cause any effects in reducing the erosion severity when injected at 18 h before cold-restraint stress. Injection of dexamethasone at 24 h before stress resulted in an increase of severity of the stress-induced gastric erosions (Fig. 2). Therefore, the data presented on Fig. 2 demonstrate transformation of gastroprotective action of dexamethasone to proulcerogenic one.

After 24 h fasting and also 3 h stress action the control groups had low blood glucose levels (Fig. 2). The pretreatment with 1 mg/kg of dexamethasone significantly reduced the blood glucose level observed 3 h after the onset of stress comparing those of control animals. Further 10-fold increase in dexamethasone dose to 10 mg/kg did not cause further potentiation the protective hormonal effect on the gastric mucosa: there were no significant differences in values of mean area of gastric erosions between the groups pretreated by dexamethasone at a dose 1 mg/kg (0.8±0.4 mm²) and 10 mg/kg (0.4±0.2 mm²). A dose of 1 mg/kg has been selected for the time-dependent study.

Fig. 1. Dose-dependent effect of dexamethasone on cold-restraint-induced gastric erosions and blood glucose level in rats. Fasting animals were given a single intraperitoneal injection of dexamethasone in doses of 0.01, 0.1, 1 mg/kg or its vehicle 1 h before the stress. Area of gastric erosions and blood glucose level were estimated 3 h after the onset of stress. Data are presented as the mean±SE from 6-12 rats/group. Significant difference at P<0.05: * from control (C, vehicle).

Fig. 2. Time-dependent effect of dexamethasone on cold-restraint-induced gastric erosions and blood glucose level in rats. In the time-dependent investigation, we varied the time interval between dexamethasone and the onset of stress. Fasting animals were given a single intraperitoneal injection of dexamethasone at a dose of 1 mg/kg at various time points: 1, 12, 18, and 24 h before the stress. Area of gastric erosions and blood glucose level were estimated 3 h after the onset of stress. Data are presented as the mean±SE from 6-12 rats/group. Significant difference at P<0.05: * from corresponding control; # from all groups. Here and in all further figures: white columns represent vehicle-treated groups and black columns represent the groups treated by dexamethasone.
by dexamethasone (1 mg/kg) resulted in maintenance of blood glucose levels observed 3 h after the onset of stress in fasted rats. Blood glucose levels of dexamethasone-pretreated groups in all time points presented were significantly higher of those in corresponding control groups. Maximal blood glucose levels after dexamethasone pretreatment was observed in the case of the hormone injection 24 h before cold-restraint (Fig. 2). There were no differences in the lost of body weight during fasting between control and dexamethasone-pretreated rats when dexamethasone was injected 1 h before stress. In the same time, in the case of dexamethasone injection 12, 18, and 24 h before stress the lost of body weight during fasting in dexamethasone-pretreated rats became significantly larger comparing of that of corresponding control (Fig. 3). Although dexamethasone injection resulted in decrease in thymus weight starting from 1 h time point, nevertheless the fall of the thymus weight progressed in the case of dexamethasone injection 24 h before stress (Fig. 3).

In control rats, cold-restraint stress induced plasma corticosterone rise which was still high in 3 h after the onset of stress (Fig. 4). Dexamethasone (at a dose of 1 mg/kg) prevented the stress-induced corticosterone rise in the case of its injection 12, 18, and 24 h before stress (Fig. 4). Minimal corticosterone blood level observed in 18 h after dexamethasone injection: the cold-restraint-induced corticosterone level in this time point was significantly lower comparing those in all other time point and even significantly lower (p<0.05) than basal corticosterone level being 7.7±1.6 µg/dl.

Additionally to the time-dependent study in which dexamethasone was injected 1, 12, 18, and 24 h before the onset...
of cold-restraint (Fig. 2 and 3), we have also performed similar investigations with dexamethasone injection 3, 5, and 7 days before the stress.

The effects of dexamethasone treatment (at a dose of 1 mg/kg) 1, 3, 5, and 7 days before cold-restraint on the stress-induced gastric erosions, blood glucose levels, thymus and spleen weight are presented on Figs 5-6. Dexamethasone still caused aggravation of cold-restraint-induced gastric erosions in the case of its injection 3, and 5 days before stress (Fig. 5). There were no significant differences in values of mean area of gastric erosions between the groups pretreated by dexamethasone 1 and 3 days before stress. In the same time, there were significant differences (P<0.05) in values of mean area of gastric erosions between the groups pretreated by dexamethasone 1 day and 5-7 days before stress: in 5 and 7 days after dexamethasone pretreatment the mean gastric erosion area was lower than that in 1 day. Moreover, in 7 days after dexamethasone pretreatment there were no significant differences in values of mean area of gastric erosions between control and dexamethasone-pretreated groups (Fig. 5). Therefore, dexamethasone did not aggravate indomethacin-induced gastric erosions in the case of its injection 7 days before stress.

The ability of dexamethasone in maintaining of blood glucose levels in fasted stressed rats disappeared in 3, 5, and 7 days after the hormone injection (Fig. 5). In 3 days after dexamethasone pretreatment normal cold-restraint-induced corticosterone rise was appeared (Fig. 4). In 3, 5 and 7 days after dexamethasone injection there were no significant differences in values of mean stress-induced corticosterone levels between dexamethasone- and vehicle-pretreated groups (Fig. 4).

Fig. 5. Time-dependent long-lasting action of dexamethasone on cold-restraint-induced gastric erosions and blood glucose levels in rats. The animals were given a single intraperitoneal injection of dexamethasone at a dose of 1 mg/kg at various time points: 1, 3, 5, and 7 days before cold-restraint. Area of gastric erosions and blood glucose levels were estimated 3 h after the onset of stress. Data are presented as the mean±SE from 12 rats/group. Significant difference at P<0.05: * from corresponding control; # from the group treated by dexamethasone "1 day".

Fig. 6. Time-dependent long-lasting action of dexamethasone on thymus and spleen weight in rats. The animals were given a single intraperitoneal injection of dexamethasone at a dose of 1 mg/kg at various time points: 1, 3, 5, and 7 days before cold-restraint. Thymus and spleen weight were estimated 3 h after the onset of stress. Data are presented as the mean±SE from 12 rats/group. Significant difference at P<0.05: * from corresponding control; # from the group treated by dexamethasone "1 day".
Dexamethasone injection resulted in 7 day-lasting (at least) decrease in thymus weight with a tendency to attenuation of this decrease at the 7th day (Fig. 6). However, negative action of dexamethasone on spleen weight significantly attenuated in time and, finally, in 5 and 7 days after dexamethasone pretreatment we already observed a similar spleen weight in both control and dexamethasone-pretreated rats (Fig. 6). Similarly, negative effect of dexamethasone on body weight also significantly attenuated in time and, finally, in 5 and 7 days after dexamethasone pretreatment we already observed a similar increase in body weight (between the onset of dexamethasone and fasting) in both control and dexamethasone-pretreated rats (Fig. 7).

DISCUSSION

The results obtained demonstrate that single injection of dexamethasone at a dose of 1 mg/kg may attenuate or aggravate cold-restraint-induced gastric erosions depending on the duration of its action before the stress. Short-lasting (1-12 hours) action of dexamethasone attenuated the stress-induced gastric ulceration. However long-lasting (more 12 hours) dexamethasone action resulted in an aggravation of cold-restraint ulceration. Both short- and long-lasting dexamethasone actions resulted in maintenance of blood glucose level in fasted stressed rats. Dexamethasone-induced long-lasting maintenance of blood glucose level accompanied with the signs of catabolic effects preceded the transformation of gastroprotective action of dexamethasone to its proulcerogenic effect.

The present results are consistent with our previous data about the similar dual effect of dexamethasone on indomethacin-induced gastric erosions (17). Interestingly enough that in both, cold-restraint or indomethacin, models the time point of 18 h could be considered as the point of transformation of gastroprotective action of dexamethasone to proulcerogenic one. The present and the previous data (17) are similar in every respect including short- and long-term maintenance of blood glucose level and catabolic effects. The coincidence of the results reflects general character of dexamethasone effects and heavily supports the hypothesis.

In both studies we verified the hypothesis investigating the effects of exogenous glucocorticoid and dexamethasone was selected for this aim as synthethic long-acting glucocorticoid. Stress and indomethacin, one of nonsteroidal anti-inflammatory drugs, were selected as ulcerogenic stimuli because of both of them are considered as most significant ulcerogenic factors in human (18-20).

Taking into consideration that action of exogenous glucocorticoids on the gastric mucosa is depended on the dose (3, 21) first, we investigated dose-dependent effects of dexamethasone. Surprisingly, dexamethasone, even at pharmacological dose 10 mg/kg protected the gastric mucosa against stress-induced injury, at least, during first hour of its action. Because of the aim of our study, here we are concentrated on the idea about maintaining glucose homeostasis as a reason of gastroprotective action of dexamethasone. However, other mechanisms may contribute to a beneficial action of dexamethasone on gastric mucosal integrity. Membrane-stabilizing effect of dexamethasone may be part of gastroprotective dexamethasone action (22).

Because dexamethasone at the dose of 1 mg/kg decreased the gastric erosion area and maintained blood glucose level in fasted stressed rats (in the case of its injection 1 h before the onset of cold-restraint) this dose has been selected for the next step, time-dependent study. In time-dependent study the gradual transition of gastroprotective action of dexamethasone to proulcerogenic effect has been showed. The results obtained suggest that manifestation of gastroprotective or ulcerogenic action of glucocorticoids used at the same dose may be dependent very much on the time interval between the hormonal injection and onset of ulcerogenic stimulus. Prolongation of glucocorticoid action may lead to enhancement of gastric mucosal susceptibility to ulcerogenic action of cold-restraint (in the present study) or indomethacin (17).

Excessive exogenous glucocorticoids can worsen glycemic control in patients and experimental animals due to their effects on glucose metabolism (23). The mechanisms through which glucocorticoids can induce hyperglycemia are many. Glucocorticoids promote hepatic gluconeogenesis, degradation of proteins to free amino acid in muscle, and lipolysis. In additional, they decrease peripheral insulin sensitivity and inhibit pancreatic insulin production and secretion (13). Chronic treatment with synthetic glucocorticoids like dexamethasone has been associated with insulin resistance. About 30% of patients who have insulin resistance eventually develop type 2 diabetes. On the base of these facts it was reasonable to assume that long-lasting action of dexamethasone may lead to uncontrollable long-lasting production of glucose through hepatic gluconeogenesis at the expense of the body resources because of catabolic effects. The results obtained confirm this possibility. Dexamethasone-induced long-lasting maintenance of blood glucose level in fasted rats was accompanied by an increase in the lost of body weight during fasting. It should be note that dexamethasone-induced increase in the lost of body weight during fasting preceded the appearance of its ulcerogenic action. Thymus weight was used as another marker of dexamethasone-induced catabolic effects. It is known that glucocorticoids at

**Fig. 7.** Effect of dexamethasone on the body weight in rats. The body weight was examined before dexamethasone injection and before the onset of fasting. The animals were given a single intraperitoneal injection of dexamethasone at a dose of 1 mg/kg at various time points: 3, 5, and 7 days before cold-restraint. Data are presented as the mean±SE from 12 rats/group. Significant difference at P<0.05: * from corresponding control group.
pharmacological doses tend to kill off many of the thymus cells. This phenomenon is the basis for the immunosuppressive use of glucocorticoids (24). It was shown that dexamethasone accelerates the rate of apoptosis in thymocytes (25). In distinguish from the body weight changes the thymus weight changes started earlier. These findings are in agreement with the data of literature showed that metabolic glucocorticoid effects in thymus cells evolve more rapidly comparing those in other target cells. The most prominent effect of glucocorticoid in thymus cells is a large inhibition of glucose transport that reaches 25-30% by about 30 min after the hormone addition. The metabolic inhibitions followed by cell destruction (24).

Dexamethasone treatment inhibited cold-restraint-induced corticosterone production: the stress-induced corticosterone rise in a blood was prevented in the case of dexamethasone injection 12, 18, and 24 h before stress. Minimal corticosterone blood level, observed in 18 h after dexamethasone injection, preceded the appearance of proulcerogenic action of dexamethasone. These data are in agreement with our results obtained in the indomethacin model. Accordingly to our previous data deficiency of corticosterone potentiates cold-restraint- and indomethacin-induced gastric erosion formation (1, 4, 8, 9). It is quite possible that simultaneous corticosterone deficiency (as result of single hormonal injection with sharp withdrawal of "the therapy") and consequences of disturbances of carbohydrate metabolism, observed in the present as well as in the previous our study, (17) contributed together to proulcerogenic effect of long-lasting dexamethasone treatment.

We prolonged our study till the 7th day to clarify the questions how long dexamethasone effects may be continued and whether they are reversible. It was found that the dexamethasone-induced proulcerogenic action was continued till the 5th day and then, on the 7th day was disappeared. The restoration of stress-induced corticosterone production which preceded the restoration of normal susceptibility of the gastric mucosa to ulcerogenic action of cold-restraint may contribute to this event. The gradual restoration of normal body and spleen weight is a good symptom of reversibility dexamethasone-induced catabolic effects. Disappearance of dexamethasone-induced maintenance of blood glucose level preceded the restoration of normal body and spleen weight.

In our experimental situations the transformation occurred 18 h after dexamethasone administration, but it is clear that in general this time interval depends on many factors, including a kind of glucocorticoid and its dose, a specificity of situation. As far back as 1950 it was noted on the base of clinical observations that it needs at least 5-7 days of corticosteroids use before appearance of ulcer symptoms (26). One of the principles for minimizing undesirable side effects of glucocorticoid therapy is "keep treatment as short as possible, since treatment lasting 5 to 7 days shows fewer side effect" (27). It is more often glucocorticoid-induced ulcer symptoms appeared after much more long hormonal treatment. Our present as well as previous (17) data allow assume that glucocorticoid-induced disturbance of carbohydrate metabolism regulation, which needs time for developing, contribute to appearance of ulcer symptoms after long-lasting hormonal therapy. It means that control of glucose regulation and its correction in case of need may be considered as useful approach minimizing side ulcerogenic effect of glucocorticoid therapy.

Biphasic, proulcerogenic and mucosal protective, action of prednisolone was investigated earlier in rats (3). To demonstrate both effects of prednisolone, three different gastric lesion models were used. It was shown that prednisolone has proulcerogenic action at higher doses and mucosal protective effects at lower doses and was concluded that the mechanisms of these actions may involve the inhibition of prostaglandin synthesis and the decreased vascular permeability (3). In distinguish from that study (3) we demonstrated the dual effects of dexamethasone with gradual transition of gastroprotective effect to proulcerogenic in the same model (cold-restraint- or indomethacin-induced ulceration) with the same dose but in the different time points after dexamethasone administration. We showed that both, gastroprotective and proulcerogenic, effects of dexamethasone may be related with its action on carbohydrate metabolism regulation. Further investigation of detailed mechanisms of the findings presented is the task of our future studies. We take into consideration other, additional, possibilities for explanation of ulcerogenic dexamethasone action on the gastric mucosa. One of them in general is inhibition of prostaglandin synthesis (28). However, it is worth to mention that in our present and previous studies we have got the similar results in two different ulcerogenic models regarding prostaglandin content.

In conclusions, our findings suggest that glucocorticoid-induced long-lasting maintenance of blood glucose levels accompanied with the signs of their catabolic effect and dexamethasone-induced corticosterone deficiency may be responsible, at least partly, for the transformation of gastroprotective action of glucocorticoids to their proulcerogenic effect.


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