High Sensitivity of Gastric Mucosa to Ulcerogenic Effect of Indomethacin in Rats with Diabetes

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One week after injection of streptozotocin (60 mg/kg intravenously), rats developed diabetes associated with a significant increase of gastric mucosa sensitivity to the ulcerogenic effect of indomethacin (35 mg/kg subcutaneously). Since potentiation of the ulcerogenic effect of indomethacin was observed only in rats subjected to fasting before drug injection, we hypothesize that this effect was caused by a drop of high glucose level in the blood after fasting.

Key Words: *diabetes; glucose; indomethacin; gastric erosion; corticosterone*

The defense mechanisms of the gastric mucosa are disordered in experimental diabetes induced by injection of streptozotocin [12]. This increases its sensitivity to such ulcerogenic exposures as fasting [9,12], cooling [8], ischemia–reperfusion of the stomach [13], and inhibits healing of gastric mucosa lesions [3,7]. The destructive effect of diabetes on the stomach was observed not before 3-5 weeks after streptozotocin injection. Indomethacin is a highly prevalent ulcerogenic factor; the contribution of diabetes to gastric mucosa sensitivity to the ulcerogenic effect of indomethacin has never been studied.

Diabetes is associated with high basal and stress activities of the hypothalamic–pituitary–adrenocortical system (HPACS) and high blood levels of glucocorticoid hormones [3,14]. Our previous experiments showed that glucocorticoid hormones produced during stress [1,4] and during exposure to other ulcerogenic factors, including indomethacin [2,5], have a protective effects on the gastric mucosa. This effect of glucocorticoids is based on their involvement in the maintenance of blood glucose level [6].

We studied the impact of diabetes developing 1 week after streptozotocin injection for the formation of indomethacin-induced erosions in the gastric mucosa of rats and its correlation with blood glucose levels (under conditions of fasting or without it) and with HPACS activity.

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MATERIALS AND METHODS

Experiments were carried out on male Sprague-Dawley rats weighing 300 g. One week before the experiment, the rats were acclimatized under standard laboratory vivarium conditions at 20-22°C, 12h:12 h light:darkness regimen (light from 8.00 to 20.00), and free access to water and food. Experiments were carried out after 24-h fasting or without it. Diabetes was induced by intravenous injection of streptozotocin (60 mg/kg; 2 ml/kg; Sigma). Controls were injected with the solvent (citrate buffer, pH 6.5). Water consumption as an indicator of diabetes development was daily measured throughout a week.

One week after injection of streptozotocin or its solvent, the rats were injected with indomethacin in the ulcerogenic dose of 35 mg/kg. Indomethacin (Sigma) suspension was prepared in saline with a droplet of Twin-80 (Theodor Schuchardt). Indomethacin or saline with Twin (control) was injected subcutaneously (5 ml/kg). Four hours after indomethacin injection, the animals were decapitated, the stomach, thymus, adrenals, and blood for measurements of corticosterone and glucose levels were collected. The area of gastric lesions was evaluated using Image J software. All ani-

mals were weighed before streptozotocin and 1 week after it and percent weights of the thymus and adrenals (per 100 g body weight) were evaluated.

Glucose content was measured in a blood drop using One Touch Ultra test strips. Activity of HPACS was evaluated by blood corticosterone level and by adrenal reaction to ACTH (Sigma) *in vitro*. For *in vitro* experiments, the isolated adrenals were cleansed from the connective tissue in the cold, half of the adrenal after weighing was plunged in the incubation medium (1 ml). After 1-h incubation, specimens for measurements of basal corticosterone in the medium were collected, after which ACTH (0.04 U) was added and after 15, 30, and 45 min specimens were collected for evaluation of the adrenal reactivity. Corticosterone levels in the plasma and incubation medium were measured by the microfluorometric method.

All data were statistically processed. The areas of gastric mucosa erosion were compared using nonparametric Mann–Whitney test, other parameters were analyzed by Student's *t* test.

RESULTS

The rats developed diabetes 1 week after streptozotocin injection, which was shown by a significant elevation of blood glucose level (up to 400 mg/dl; Fig. 1, *b*), increase of water consumption (Fig. 1, *a*), and body weight loss (Fig. 1, *c*). Injection of indomethacin to non-fasting diabetic rats did not change blood glucose level, which remained as high as before indomethacin. Indomethacin injection to fasting (24 h) rats led to a drop of blood glucose level after 4 h. In controls (injection of streptozotocin solvent), a slight but significant reduction of blood glucose level in comparison with that in non-fasting controls developed under these conditions. Hence, fasting reduced blood glucose levels in both groups, as a result of which glucose levels in these groups of animals were the same (Fig. 1, *b*).

Our findings indicated that diabetes did not lead to injuries in the rat gastric mucosa. Injection of indomethacin to non-fasting rats caused small erosions of the gastric mucosa, their size did not differ in animals injected with streptozotocin and in the controls (Fig. 2, *a*). These data indicated that the sensitivity of the gastric mucosa to the ulcerogenic factor in non-fasting diabetic rats did not differ from that in non-fasting controls. Fasting stimulated sensitivity of the gastric mucosa to indomethacin, this effect in diabetic rats was more pronounced than in controls (Fig. 2, *a*). The observed potentiation of the ulcerogenic effect of indomethacin was presumably caused by a drop of blood glucose level after 24-h fasting: these animals developed a virtually 30-fold increase in the mean area of erosion (from 1.12 to 34.2 mm^2) in comparison with

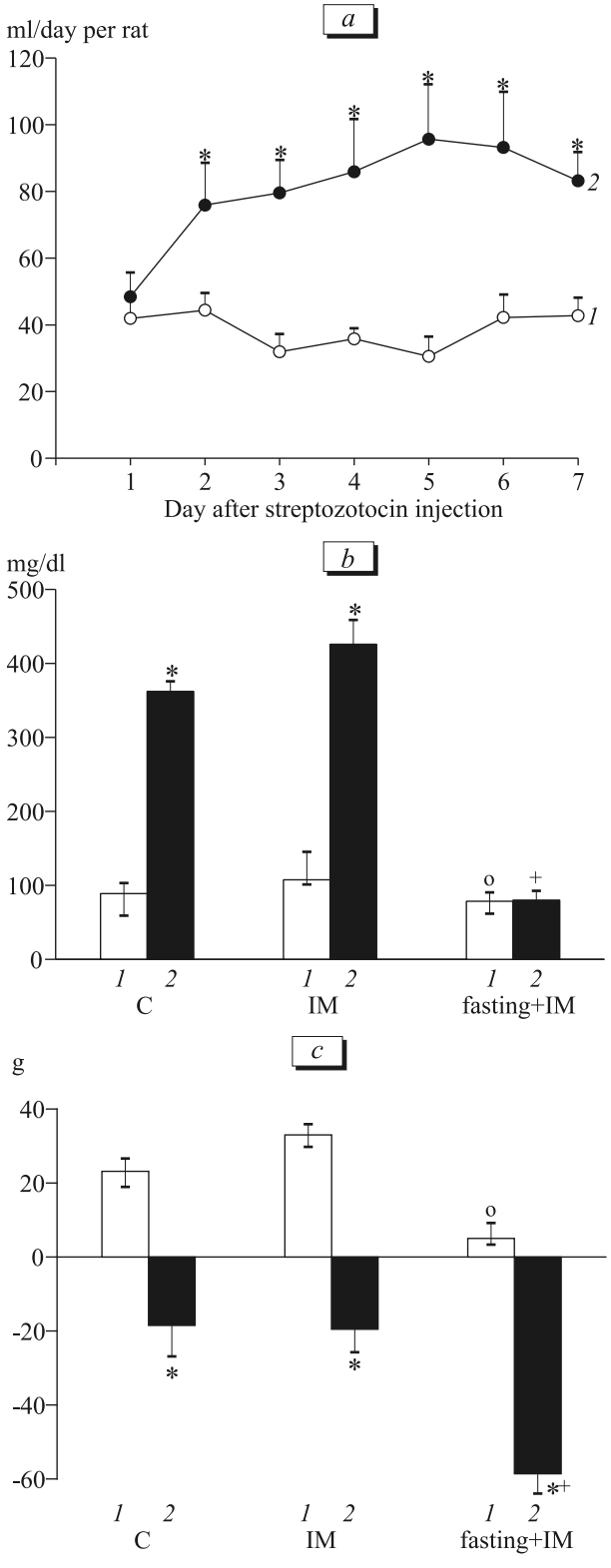

Fig. 1. Effects of streptozotocin on water consumption (*a*), blood glucose level (*b*), and changes in body weights (*c*) 1 week after injection. Here and in Figs. 2 and 3: *1*) streptozotocin solvent; *2*) streptozotocin. IM: indomethacin; C: indomethacin control. $p<0.05$ in comparison with: **1* in the same group; °*1* in IM group; +*2* in IM group.

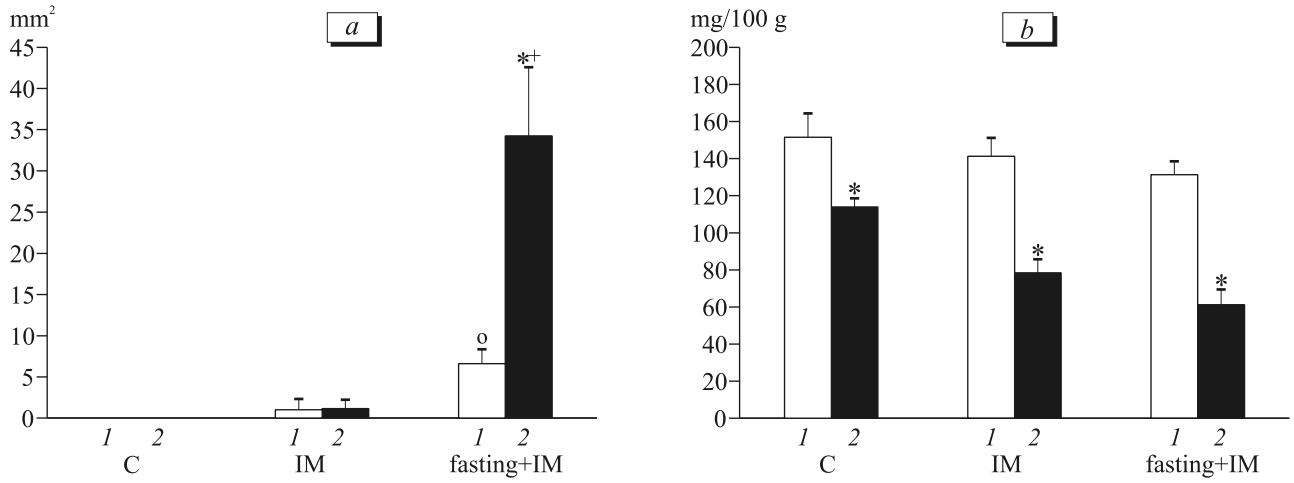

Fig. 2. Effect of diabetes caused by streptozotocin injection on the formation of erosions in the gastric mucosa (*a*) and thymus weights (*b*).

non-fasting diabetic rats, and a significant reduction of glucose level after fasting (from 425.7 to 79.8 mg/dl). Presumably, potentiation of the ulcerogenic effect of indomethacin on the stomach under these conditions was caused by the degree of glucose level drop, rather than low blood glucose level after fasting (in the diabetic rats it decreased to a level observed in control animals; Fig. 1, *b*). A drop of blood glucose

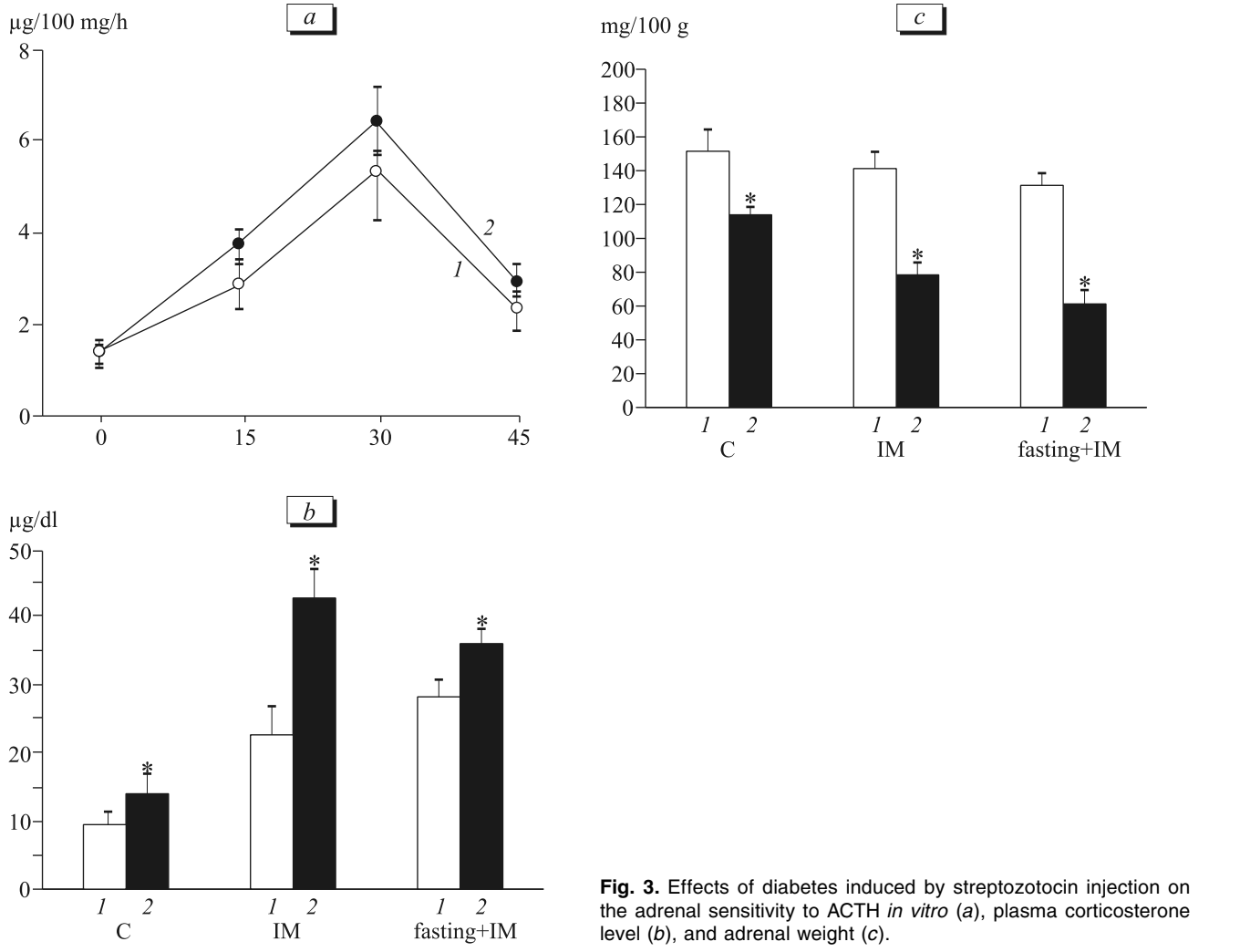

Fig. 3. Effects of diabetes induced by streptozotocin injection on the adrenal sensitivity to ACTH *in vitro* (*a*), plasma corticosterone level (*b*), and adrenal weight (*c*).

level could lead to stimulation of gastric motoricity, a key pathogenetic factor of indomethacin-induced ulcer formation [7] and hence, stimulate the sensitivity of the gastric mucosa to indomethacin in diabetic rats. Presumably, long-lasting (1 week) disorders in glucose homeostasis could also promote liability of the gastric mucosa to increase of its sensitivity to subsequent exposure.

Fasting of rats with diabetes in our experiments did not lead to development of visible erosions in the stomach, which was in line with the data of other scientists, according to which fasting proper could lead to injuries in the gastric mucosa of diabetic rats not before 3 weeks after streptozotocin injection [9,12]. However, in contrast to published data, according to which the sensitivity of the gastric mucosa to ulcerogenic factors increased only 3-5 weeks after streptozotocin injection [11,13], our results indicated that diabetes enhanced gastric mucosa sensitivity to indomethacin injected in an ulcerogenic dose to rats after 24-h fasting as early as just 1 week after streptozotocin injection.

Blood corticosterone level in diabetic rats was elevated 4 h after indomethacin injection in comparison with the value in controls (Fig. 3, *b*). The reactivity of adrenals to ACTH *in vitro* did not change in these animals (Fig. 3, *a*), which indicated high reactivity of the HPACS in general. The increase of the adrenal weight (Fig. 3, *c*) and reduction of thymus weight (Fig. 2, *b*) in diabetic rats also indicated stimulation of the HPACS under these pathological conditions. The data on HPACS stimulation soon after diabetes onset were in line with our previous data [14] and with other reports [3,10]. It was known than HPACS was involved in the maintenance of blood glucose level: hypoglycemia led to its stimulation and hence, to rapid elevation of the levels of glucocorticoid hormones and subsequent normalization of blood glucose level. Elevation of glucocorticoid levels in diabetes under conditions of hyperglycemia presumably reflected the disorders in the normal "interrelationships" between HPACS and glucose metabolism. Chronic stimulation of HPACS in diabetes could augment disorders of glucose homeostasis and enhance vulnerability of the gastric mucosa in rats. Verification of this hypothesis is the matter of future studies.

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