Mechanisms by which endogenous glucocorticoid protects against indomethacin-induced gastric injury in rats

LUDMILA FILARETOVA, AKIKO TANAKA, TOHRU MIYAZAWA, SHINICHI KATO, AND KOJI TAKEUCHI

Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Kyoto 607-8414, Japan

Received 17 May 2002; accepted in final form 26 July 2002

Filaretova, Ludmila, Akiko Tanaka, Tohru Miyazawa, Shinichi Kato, and Koji Takeuchi. Mechanisms by which endogenous glucocorticoid protects against indomethacin-induced gastric injury in rats. Am J Physiol Gastrointest Liver Physiol 283: G1082–G1089, 2002.—We investigated the mechanisms underlying the protective action of glucocorticoids against indomethacin-induced gastric lesions. One-week adrenalectomized rats with or without corticosterone replacement (4 mg/kg sc) were administered indomethacin (25 mg/kg sc), and gastric secretion (acid, pepsin, and mucus), motility, microvascular permeability, and blood glucose levels were examined. Indomethacin caused gastric lesions in sham-operated rats, with an increase in gastric motility and microvascular permeability as well as a decrease in mucus secretion. Adrenalectomy significantly worsened the lesions and potentiated these functional disorders. Glucose levels were lowered by indomethacin in sham-operated rats, and this response was enhanced by adrenalectomy. The changes observed in adrenalectomized rats were prevented by supplementations of corticosterone at a dose mimicking the indomethacin-induced rise in corticosterone, whereas the protective effect of corticosterone was attenuated by RU-38486, a glucocorticoid receptor antagonist. We conclude that the gastroprotective action of endogenous glucocorticoids may be provided by their support of glucose homeostasis and inhibitory effects on enhanced gastric motility and microvascular permeability as well as maintaining the production of mucus.

gastric lesion; gastric microvascular permeability; gastric motility; mucus; acid and pepsin secretion

INDOMETHACIN, a potent anti-inflammatory drug (NSAID), was introduced in 1963 for the treatment of rheumatoid arthritis and related diseases. Eight years later, it was demonstrated that indomethacin and aspirin inhibit prostaglandin (PG) biosynthesis (6, 42). A reduction in the biosynthesis of PG through inhibition of cyclooxygenase (COX) is the pharmacological background to both the anti-inflammatory action (42) and the harmful side effects of indomethacin, as well as other NSAIDs (14). The gastrointestinal side effects of NSAIDs, especially in the stomach, are one of the more serious complications in patients taking these drugs (14, 44). Indeed, indomethacin shows potent ulcerogenic action in experimental animals (38, 44). The mechanism by which indomethacin induces gastric injury is generally considered to involve depletion of PGs, yet it has proven more complicated than expected and involves multiple, closely interacting elements such as gastric hypermotility, microcirculatory disturbances, neutrophil-endothelial cell interactions, and superoxide radicals, in addition to PG deficiency (39, 44).

NSAID-induced gastropathy has advanced investigations directed at ways to achieve a reduction in their ulcerogenic properties, and some have been successfully incorporated into clinical practice. During a deficiency of PG, other defensive mechanisms might operate to attenuate NSAID-induced gastropathy, and knowledge of the compensatory mechanisms could be a basis for the creation of new clinical approaches. The results of our previous studies (7, 8, 10, 36) suggested that endogenous glucocorticoids are gastroprotective substances, which play a role as natural defensive factors in maintaining the integrity of the gastric mucosa during NSAID therapy. We have shown that indomethacin and aspirin at ulcerogenic doses induce a rise in corticosterone, which helps the gastric mucosa to resist the harmful actions of these ulcerogenic agents (7, 8, 10). The beneficial effect of an acute elevation in glucocorticoids during NSAID action is the opposite of the well-known ulcerogenic action of long-lasting glucocorticoid treatment (14, 46). However, the findings support the idea that glucocorticoids released during activation of the hypothalamic-pituitary-adrenocortical (HPA) axis caused by various ulcerogenic stimuli act as a gastroprotective hormone (7, 8, 10) but not as an ulcerogenic hormone as has generally been accepted for some decades.

The present study was designed to clarify the mechanisms underlying the gastroprotective action of glucocorticoids against indomethacin-induced injury. With this aim, we investigated the effects of glucocorticoid deficiency caused by adrenalectomy followed by corticosterone replacement on various parameters of gastric function.
GASTROPROTECTION BY ENDOGENOUS CORTICOSTERONE

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Nippon Charles River, Shizuoka, Japan), weighing 280–300 g, were housed in groups of five and acclimatized to standard laboratory conditions (12:12-h light-dark cycle, temperature 20 ± 1°C). The animals were kept in cages with raised mesh bottoms to prevent coprophagy and were deprived of food but allowed free access to tap water for 18 h before the experiment. The experimental procedures employed in the present study were all approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University.

Experimental Protocol

The animals were given indomethacin (25 mg/kg sc), and five parameters of gastric function (gastric motility, gastric microvascular permeability, gastric mucus content, acid and pepsin secretion, and blood glucose level) were examined, in addition to the gastric ulcerogenic response. Each parameter was studied in a separate experiment but under identical experimental conditions. In all cases except for motility, the experiment was performed in sham-operated rats, adrenalectomized rats, and adrenalectomized animals with corticosterone replacement, either with or without administration of indomethacin. Rats were adrenalectomized through a lateral incision below the last rib 1 wk before administration of indomethacin. Sham-operated rats were subjected to the same surgical procedure, but 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 (4 mg/kg) 15 min before the administration of indomethacin to adrenalectomized rats. Our previous results showed that this treatment mimics the indomethacin-induced rise in corticosterone in adrenalectomized rats and prevents the aggravation of gastric lesions in response to indomethacin in these animals (10). In addition, we evaluated whether or not the glucocorticoid antagonist RU-38486 prevents the effect of corticosterone replacement on gastric ulcerogenic and blood glucose responses to indomethacin in adrenalectomized animals. RU-38486 (20 mg/kg) was given orally 30 min before administration of corticosterone.

Evaluation of Gastric Lesions

The animals were killed under deep ether anesthesia 4 h after administration of indomethacin. Their stomachs were removed under ether anesthesia and were inflated by injecting 10 ml of 2% formalin, were immersed in 2% formalin for 10 min to fix the gastric tissue wall, and were opened along the greater curvature. Then the area (mm²) of hemorrhagic lesions developed in the corpus mucosa was measured under a dissecting microscope with a square grid (×10), were summed per stomach, and were used as a lesion score.

Measurement of Gastric Motility

Gastric motility was determined by using a miniature balloon as described in a previously published study (38). Briefly, under ether anesthesia the balloon and the support catheter were placed into the stomach through an incision of the forestomach. The animals were kept in Bollman cages, and the gastric motility was continuously monitored on a recorder (model 586; Hitachi, Tokyo, Japan) as intraluminal pressure recordings by using a pressure transducer (Model 151-T; Narco Telecare, Houston, TX) and a polygraph device (Model 6M-72; San-ei, Tokyo, Japan) after complete recovery from anesthesia. After basal motility was well stabilized, the sham-operated rats and half of the adrenalectomized rats were administered with indomethacin, and another half of adrenalectomized animals were injected with corticosterone, 15 min later all rats were administered indomethacin, and then the motility was measured for 4 h. A quantitative analysis of the gastric motility was made by measuring the area of motility changes in a recording sheet over a 15-min period using NIH Image 1.61 (National Institutes of Health, Bethesda, MD), and the data were expressed as a motility index (arbitrary units).

Determination of Microvascular Permeability

The microvascular permeability was measured as the amount of dye (Evans blue) extravasated in the gastric mucosa, as described in a previous study (34). Four hours after administration of indomethacin, the animals were killed under deep ether anesthesia. In each case, 1 ml of 1% Evans blue (wt/wt) was injected intravenously 30 min before death. To remove a dye from the gastric vessels, the animals were killed by bleeding from the descending aorta, the stomachs were removed, and the amount of dye that had accumulated in the corpus mucosa in 30 min was measured. The extraction of dye was performed according to the method described by Katayama et al. (16). The absorbance of each sample was measured at 620 nm on a Hitachi spectrophotometer (U1100; Mito), and the amount of dye recovered from the corpus mucosa was expressed in micrograms per gram of wet tissue.

Determination of Gastric Mucus Contents

Four hours after administration of indomethacin, the animals were killed under deep anesthesia and their stomachs were removed and opened along the greater curvature. The mucus contents, including soluble and adherent mucus gel, were measured as described by Azumi et al. (1). Briefly, the gastric mucosa was immersed in 10 ml PBS containing 2% (wt/vol) N-acetylcysteine and gently stirred with a magnetic stirrer for 5 min at room temperature. The mucosa was then gently rinsed with 10 ml PBS twice, and the solution recovered from these rinsing steps was pooled as an adherent mucus sample. After lyophilization, the powdered samples were dissolved in 3 ml of 50 mM Tris-HCl (pH 7.2) containing 2% Triton X-100, homogenized with a Polytron tissue homogenizer (IKA, Yokohama, Japan), and centrifuged at 10,000 g for 30 min at 4°C. An aliquot of the supernatant was applied to a column of Sepharose 2B (Amersham Pharmacia Biotech, Uppsala, Sweden) every 50 cm and eluted with 50 mM Tris-HCl (pH 7.2) containing 2% Triton X-100. The void volume fraction was collected, and the hexose content was measured as mucin by the phenol-sulfuric acid method, using galactose as the standard (5).

Measurement of Gastric Secretion

Gastric secretion was measured in pylorus-ligated rats. Under ether anesthesia, the abdomen was opened and the pylorus was ligated, and the animals were allowed to recover from the anesthesia. Four hours later, the animals were killed by deep ether anesthesia, the stomach was removed, and then the gastric contents were collected. After centrifugation for 10 min at 3,000 rpm, each sample was measured for volume and titrated with 100 mM NaOH to pH 7.0 by using an automatic titrator (Comite-8; Hiranuma, Mito, Japan) for titratable acidity. The pepsin concentration was determined according to a previous paper (35) and expressed as milligrams of pepsin per milliliter.
Determination of Blood Glucose Levels

Levels of blood glucose were measured before and 4 h after administration of indomethacin. A drop of blood was taken under ether anesthesia from the tail vein through a small cut, and the glucose level was determined with the aid of a MediSense system (Abbott Laboratories, Bedford, MA) using the blood glucose sensor electrode.

Statistical Analysis

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

RESULTS

Alteration of Gastric Ulcerogenic Response to Indomethacin in Adrenalectomized Rats

Indomethacin (25 mg/kg sc) produced hemorrhagic erosion in the glandular stomach in sham-operated animals, the lesion score being 2.2 ± 0.8 mm². The gastric ulcerogenic response induced by indomethacin was markedly aggravated in adrenalectomized rats, and the lesion score was about five times the value observed in sham-operated animals (Fig. 1). Corticosterone (4 mg/kg sc) injected into adrenalectomized rats shortly before indomethacin significantly prevented the erosion-promoting effect of adrenalectomy. The glucocorticoid antagonist RU-38486 reversed the protective effect of corticosterone replacement on indomethacin-induced gastric lesions. Adrenalectomy by itself did not cause any damage to the gastric mucosa.

Alteration of Gastric Functional Responses to Indomethacin in Adrenalectomized Rats

Gastric motility. The stomach in sham-operated rats contracted at a frequency of 17.3 ± 0.3/15 min with an amplitude of 17.5 ± 2.0 cmH₂O. Subcutaneously administered indomethacin enhanced gastric motility in both sham-operated and adrenalectomized animals. However, in adrenalectomized rats the onset of the gastric motility response occurred much earlier and the degree of this response was significantly greater than in sham-operated animals (Figs. 2 and 3). The motility index became significantly larger than the basal value 1.5–2 h after administration of indomethacin.
acin in sham-operated rats, reaching about two times the basal level, whereas it increased within 45 min after the administration, reaching over five times the basal level in adrenalectomized animals. Corticosterone injected 15 min before indomethacin prevented the enhancement of gastric motility in response to indomethacin in adrenalectomized animals. Adrenalectomy alone did not affect gastric motility in terms of frequency or amplitude, resulting in no alteration in the motility index.

Microvascular permeability. Adrenalectomy alone resulted in a twofold increase in the microvascular permeability of the stomach, and this increase was prevented by a single injection of corticosterone (Fig. 4). Subcutaneously administered indomethacin caused a slight but significant increase in gastric microvascular permeability in sham-operated animals, and this response was much greater following adrenalectomy. Acute replacement of corticosterone in adrenalectomized rats prevented the increase in gastric microvascular permeability in response to indomethacin.

Gastric secretion. In sham-operated rat stomachs, the basal acid output and pepsin concentration were 122.9 ± 14.5 μeq/h and 1.2 ± 0.2 mg/ml, respectively. Adrenalectomy by itself significantly reduced both acid and pepsin secretion compared with sham-operated animals. Indomethacin did not significantly affect gastric secretion, irrespective of whether it was injected in sham-operated or adrenalectomized animals (Fig. 5). Replacing corticosterone prevented the reducing effects of adrenalectomy on both acid output and pepsin concentration, the effect on acid output being significant in the animals given indomethacin.

Secretion of mucus. The lowest gastric mucus content, significantly different from that in all other experimental groups, was observed in adrenalectomized animals 4 h after the administration of indomethacin (Fig. 6). Replacement of corticosterone reversed the decrease in gastric mucus content in adrenalectomized rats given indomethacin, and the values were significantly higher than those in the corresponding sham-operated animals. Neither adrenalectomy nor indomethacin alone significantly changed the gastric mucus content, although in sham-operated rats some tendency toward a decrease was observed after administration of indomethacin.

Fig. 4. Effect of indomethacin on gastric microvascular permeability in Sham and ADX rats with or without Cort. The animals were given vehicle or indomethacin (25 mg/kg sc) and killed 4 h later. Corticosterone (4 mg/kg sc) was given 15 min before the administration of vehicle or indomethacin. Values are means ± SE from 5–7 rats/group. *P < 0.05 vs. vehicle in Sham (*), ADX (#), and ADX + indomethacin rats ($).

Fig. 5. Effect of indomethacin on gastric acid output (A) and pepsin concentration (B) in Sham and ADX rats with or without Cort. The pylorus-ligated animals were given vehicle or indomethacin (25 mg/kg sc) and killed 4 h later. Corticosterone (4 mg/kg sc) was given 15 min before the administration of vehicle or indomethacin. Values are means ± SE from 5–10 rats/group. *P < 0.05 vs. vehicle in Sham (*), ADX (#), and ADX + indomethacin rats ($).

Fig. 6. Effect of indomethacin on gastric mucus contents in Sham and ADX rats with or without Cort. The animals were given vehicle or indomethacin (25 mg/kg sc) and the mucus content was measured 4 h later. Corticosterone (4 mg/kg sc) was given 15 min before the administration of vehicle or indomethacin. Values are means ± SE from 5–7 rats/group. *P < 0.05 vs. vehicle in Sham (*), ADX (#), and ADX + indomethacin rats ($).
Changes in Blood Glucose Level After Administration of Indomethacin

It is known that blood glucose levels are markedly decreased in adrenalectomized rats (36) or in normal rats after administration of NSAIDs (20, 37). To investigate whether changes in blood glucose levels are causally related to the increase in gastric ulcerogenic and hypermotility responses in adrenalectomized rats, we measured blood glucose levels in all experimental groups.

Levels of blood glucose were 118.0 ± 3.59 mg/dl in intact animals without any surgical manipulation, whereas they were 107.0 ± 2.18 mg/dl 1 wk after the sham operation. Adrenalectomy by itself significantly reduced these levels (Fig. 7). Indomethacin also decreased blood glucose levels in both sham-operated and adrenalectomized rats, yet the decrease was much greater in the latter. Replacing corticosterone in adrenalectomized rats totally restored the level of blood glucose to that of sham-operated animals, but the glucocorticoid receptor antagonist RU-38486 attenuated this effect.

DISCUSSION

The present study confirmed our previous results (7, 8, 10) demonstrating that endogenous glucocorticoids released in response to indomethacin increase the resistance of the gastric mucosa to the ulcerogenic action of this agent. We further suggested that the gastroprotective action of glucocorticoids against indomethacin-induced injury is accounted for by a beneficial effect on defensive factors such as the secretion of mucus and attenuating effects on pathogenic elements such as an increase in gastric motility and microvascular permeability.

We have previously reported that indomethacin caused gastric hypermotility at an ulcerogenic dose and that this response is a key to the pathogenesis of indomethacin-induced damage (36–38) and also to the mechanism for aggravation of the damage caused by adrenalectomy (36). In the present study, we confirmed that indomethacin markedly enhanced gastric motility in sham-operated rats and that this hypermotility was significantly potentiated in adrenalectomized rats, in parallel with an increase in the gastric lesion score. These results support a causal relationship between gastric hypermotility and lesion formation following administration of indomethacin. Mersereau and Hinchey (20) reported a role for the glycoprivic response in the mechanism of gastric hypermotility induced by NSAIDs. We observed in this study that blood glucose levels were lowered in adrenalectomized rats and decreased further after administration of indomethacin, suggesting a relationship between lowered blood glucose levels and enhanced gastric motility and ulcerogenic responses to indomethacin. Indeed, adrenalectomized rats given indomethacin showed minimum blood glucose levels, maximum gastric motility index values, and maximum gastric lesion score compared with other experimental groups. It should be noted that the magnitude of the fall in serum glucose levels after indomethacin was greater in sham-operated rats than in adrenalectomized animals, although the lowest values were observed in the latter. Since blood glucose levels were significantly decreased in adrenalectomized rats because of lack of glucocorticoids, this could minimize the further decreasing effect of indomethacin. A single injection of corticosterone to adrenalectomized animals, at the dose imitating the indomethacin-induced rise in corticosterone (7), restored blood glucose levels and significantly reduced gastric hypermotility and ulcerogenic responses to indomethacin, suggesting a role for glucocorticoids in gastric protection and glucose metabolism. This idea was also supported by the finding that the effects of corticosterone replacement in adrenalectomized rats were significantly antagonized by coadministration of RU-38486, a specific glucocorticoid receptor antagonist, which is known to bind with high affinity to type II glucocorticoid receptors (22) and may block the effect of glucocorticoids on glucose metabolism (13). These results are consistent with our previous findings that RU-38486 aggravated indomethacin-induced gastric lesions by antagonizing the beneficial influences of endogenous glucocorticoids released in response to indomethacin (10) and strongly suggest that lack of glucocorticoids may be a major mechanism for aggravation by adrenalectomy of indomethacin-induced gastric lesions. In addition, the present study also supports the previous findings by Urushidani et al. (41), who showed that adrenal medullectomy had no effect on indomethacin-induced gastric lesions, suggesting that the worsening effect of adrenalectomy on these lesions may be largely attributable to deficiency of glucocorticoids caused by removal of the cortex.
NSAIDs induce a decrease in blood glucose levels, probably through the uncoupling of oxidative phosphorylation (15). Hypoglycemia may lead to at least two events in the brain. An insufficient supply of glucose stimulates hypothalamic glucose-sensitive neurons (21), resulting in a vagally mediated increase in gastric motility and secretion (30). Hypoglycemia is also the major trigger for the hypothalamic events directed toward the normalization of blood glucose levels, resulting in stimulation of glucocorticoid secretion through activation of the HPA axis (45, 47). Indeed, indomethacin caused a rise in blood corticosterone levels (10) as well as a fall in blood glucose levels (37). Because glucocorticoids maintain blood glucose levels by influencing glucose homeostasis (18) and because glucose reverses the hypoglycemia-induced stimulation of hypothalamic glucose-sensitive neurons (25) and inhibits vagally mediated gastric hypermotility (2), it is reasonable to assume that supplemental glucocorticoids suppressed the enhanced gastric hypermotility caused by indomethacin in adrenalectomized rats by maintaining blood glucose levels.

The increased gastric microvascular permeability is another pathogenetic element in the formation of indomethacin-induced lesions (37, 44). It has been demonstrated that glucocorticoids have the ability to inhibit vascular permeability in animals and in humans (29, 36). It is therefore reasonable to conclude that the protective effect of corticosterone on indomethacin-induced gastric lesions is provided by their attenuative effect on the increased microvascular permeability. This idea was supported by the present findings that the microvascular permeability was markedly increased in adrenalectomized rats, irrespective of whether or not they were given indomethacin, and that this alteration was totally inhibited by supplements of corticosterone. Previous studies (39) suggested that the increase of gastric microvascular permeability in indomethacin-treated rats is caused by vascular disturbances due to abnormal contraction of the stomach wall mediated by several events, including neutrophil-endothelial interaction. It is assumed that the effect of corticosterone on the microvascular permeability is brought about by its inhibitory action on gastric hypermotility induced by indomethacin through amelioration of glucose metabolism. On the other hand, it is possible that corticosterone directly inhibits the microcirculatory disturbances by strengthening the vascular integrity (32). Indeed, the present study showed that a deficiency of glucocorticoids by itself induced a significant increase in gastric microvascular permeability in adrenalectomized rats and that this increase was totally prevented by corticosterone replacement. Glucocorticoids are known to decrease vascular permeability by inducing production of the anti-inflammatory protein vasoregulin (26, 27). Because vascular permeability is enhanced by vascular endothelial growth factor (VEGF) (3), and because the expression of VEGF is stimulated by hypoglycemia (31) and inhibited by glucocorticoids (23), it is possible that the effect of corticosterone on gastric microvascular permeability is mediated by downregulation of VEGF expression.

Gastric secretion is also an important factor in the pathogenesis of indomethacin-induced gastric lesions, inasmuch as the development of these lesions was prevented by antacids or antisecretory drugs (38). In the present study, however, both acid and pepsin output were significantly decreased in adrenalectomized rats in a corticosterone-sensitive manner. Since the severity of indomethacin-induced gastric lesions was worsened by adrenalectomy and reduced by corticosterone replacement, it is unlikely that gastric hypersecretion is involved in the mechanism underlying aggravation of these lesions in adrenalectomized rats.

The secretion of mucus, one of several defensive factors in the gastrointestinal tract (43), is another possible target for the action of glucocorticoids. In general, glucocorticoids inhibit both the production and secretion of mucus (19, 24, 28). Because glucocorticoids are released during stress, it is considered that this action is involved in the pathogenic mechanism of stress-induced ulceration. In the present study, however, neither adrenalectomy nor indomethacin alone significantly affected the secretion of mucus in sham-operated rats. Several studies reported a decrease in mucus secretion by indomethacin at a high dose (60 mg/kg) (43) or on long-term administration (12). Since indomethacin induced the release of endogenous corticosterone (7), it may be possible that the inhibitory effect of indomethacin is counteracted by corticosterone, resulting in minimal changes in mucus secretion. Alternatively, because the mucus secretion is physiologically regulated by both PG (33) and nitric oxide (4), it is possible that only inhibiting PG production is not enough to cause a profound decrease in the mucus secretion; in other words, nitric oxide acts in a compensatory role in maintaining this secretion under PG deficiency. On the other hand, the lack of change in the secretion of mucus in adrenalectomized rats may be explained by a compensatory increase in PG production due to the upregulation of COX-2 expression (9). Indeed, a marked decrease in the secretion of mucus was observed when a deficiency of both PGs and glucocorticoids was induced by administration of indomethacin in adrenalectomized rats. Of course, this decrease was completely reversed by corticosterone replacement, the value reaching even higher than that in the corresponding sham-operated animals, suggesting a role of glucocorticoids in maintaining the mucus secretion. Yet it remains unknown how glucocorticoids at physiological doses maintain mucus production. Because a glucose deficit may lead to the loss of gastric mucus glucoproteins (17), it is also possible that glucocorticoids maintain mucus production partly through glucose metabolism. Tanaka et al. (40) recently reported that glucocorticoids at physiological doses stimulate the expression of MUC5AC, the core protein of rat gastric mucus, although this expression was suppressed by pharmacological doses. These results suggest that endogenous glucocorticoids are essential for maintaining gastric mucus production.
REFERENCES


