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Protective effect of cromakalim and diazoxide, and proulcerogenic effect of glibenclamide on indomethacin-induced gastric injury [1]

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Abstract

We investigated the influences of the K^+ channel opening drugs cromakalim and diazoxide and their blocker, glibenclamide, in indomethacin-induced gastric injury in rats. Cromakalim (0.1 and 0.3 mg/kg) and diazoxide (10 and 30 mg/kg) produced dose-dependent gastroprotection at doses that were also effective on the cardiovascular system. Glibenclamide reversed their gastroprotective effects and aggravated indomethacin-induced gastric damage by its own. Cromakalim (10^{-9}–10^{-5} M) and diazoxide (10^{-9}–10^{-4} M) relaxed noradrenaline pre-contracted gastric arteries (94.59 ± 1.58% and 93.86 ± 2.99%, respectively). Their relaxant effects were inhibited by glibenclamide (10^{-5} M) but not by indomethacin (10^{-5} M) and L^G-nitro-L-arginine (10^{-4} M). Cromakalim (0.1 and 0.3 mg/kg) did not change gastric mucosal blood flow but increased the gastric mucosal vascular conductance in anaesthetized rats as measured by the hydrogen gas clearance technique. Indomethacin increased myeloperoxidase activity in the gastric mucosa, an effect which was reversed by cromakalim and diazoxide. Glibenclamide abolished their effects on myeloperoxidase activity and, alone, increased this parameter. Additionally, indomethacin caused infiltration of neutrophils which was reduced by cromakalim and diazoxide in a glibenclamide sensitive manner. The effects of cromakalim and diazoxide on mucosal myeloperoxidase activity, neutrophil infiltration and gastric injury correlated with each other. The effects of diazoxide (30 mg/kg) and glibenclamide (10 mg/kg) on blood glucose level were not correlated with their effects on gastric injury. Taken together, K^+ channel opening drugs show misoprostol-like protective effects in indomethacin-induced gastric injury which seems to be related to modulation of neutrophil function.

Keywords: Cromakalim; Diazoxide; Glibenclamide; Indomethacin-induced gastric injury; Neutrophil activation; Gastric artery

1. Introduction

Nonsteroidal antiinflammatory drugs (NSAIDs) are frequently used as antiinflammatory agents but their use is limited by gastrointestinal side effects ranging from dyspeptic symptoms to bleeding or perforation of gastric and duodenal ulcers (Fried et al., 1991). The mechanism responsible for their ulcerogenic effects is not well understood. It is generally accepted that inhibition of prostaglandin synthesis is the main defect in the occurrence of this pathology, since prostaglandins are essential factors in the regulation of mucosal homeostasis (Kauffman et al., 1980; Eberhart and Dubois, 1995). The maintenance of gastric mucosal integrity depends, among others, on the balance between vasodilator and vasoconstrictor substances. Prostaglandins and nitric oxide (NO), which are endothelial vasodilators, preserve the gastric mucosal integrity by providing adequate mucosal blood flow (Whittle et al., 1990; Lippe and Holzer, 1992; Lopez-Belmonte and Whittle, 1994) whereas, endothelin-1 and thromboxane A_2 are potent vasoconstrictors worsening gastric mucosal in-

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jury by limiting mucosal blood flow (Esplugues and Whittle, 1988; Wallace et al., 1989).

In experimental ulcer models, it has been shown that NSAIDs reduce gastric mucosal blood flow at the site of ulceration (Ashley et al., 1985; Kitahura and Guth, 1987). In vivo studies have demonstrated that NSAIDs promote leucocyte adherence along the microvascular endothelium of the gastric mucosa (Kitahura and Guth, 1987; Wallace et al., 1991; Yoshida et al., 1993). Additionally, the severity of gastric damage induced by NSAIDs can be reduced by neutrophil depletion or using monoclonal antibodies against leucocyte and endothelial adhesion molecules (Wallace et al., 1990, 1991; Wallace, 1993). Wallace et al. (1993) proposed that the adherence of neutrophils to the vascular endothelium, resulting in partial occlusion of the gastric microcirculature and free radical generation, is a critical event in the pathogenesis of NSAID-induced gastropathy.

Several studies indicate that cromakalim and diazoxide activate K_{ATP} channels in vascular smooth muscle cells leading to vasodilatation (Quast and Cook, 1989; Quayle et al., 1995). However, there is no report on the role of the activation of K^+ channels, which are functional in the regulation of vascular tone (Quast, 1993; Quayle et al., 1995), in supplying gastric mucosal blood flow. In experimental myocardial ischemia, bimakalim, a K_{ATP} channel opener, has been shown to reduce the infarct size and myeloperoxidase activity in the ischemic area in dogs (Auchampach and Gross, 1994; Mizumura et al., 1995). However, only little is known on the effects of K^+ channel opening drugs in gastric injury (Goswami et al., 1997).

The objectives of the present study were 2-fold. First, we determined whether pretreatment with two K^+ channel openers, namely, cromakalim and diazoxide would protect the gastric mucosa from indomethacin-induced injury and compared their effects with that of misoprostol, a prostaglandin E_1 analogue, which is currently used in therapy. Second, we attempted to clarify the underlying mechanism of the gastroprotective effects of the K^+ channel openers.

2. Materials and methods

2.1. Indomethacin-induced gastric lesions

Experiments were performed in male rats weighing 200–250 g that were fasted for 22 h with free access to water. Rats were kept in individual cages with raised mesh bottoms to avoid coprophagia in a light controlled environment. Experiments all started approximately at the same time because of the known circadian variation of mucosal defence (Larsen et al., 1994).

Indomethacin was administered orogastrically in a dose of 30 mg/kg. Diazoxide (10 and 30 mg/kg), cromakalim (0.1 and 0.3 mg/kg), glibenclamide (10 mg/kg) and atropine (10 mg/kg) were administered intraperitoneally, whereas cimetidine (100 mg/kg) and misoprostol (200 μg/kg) were given orally, 30 min before indomethacin treatment. Six hours after indomethacin treatment, rats were killed by a sharp blow on the head and rapidly exsanguinated. Their stomachs were removed and opened along the small curvature, rinsed with tap water and examined for gastric mucosal hemorrhagic lesions.

The extent of macroscopically visible gastric hemorrhagic lesions was scored by an observer unaware of the treatment. The length of each lesion was measured in millimeters and the lesion score was calculated as sum of the lengths of all hemorrhagic lesions. Five petechial lesions were scored as 1 mm.

In some experiments animals received only the vehicles of the agents in order to assess potential solvent effects.

2.2. Determination of blood glucose levels

Blood samples for glucose analysis were obtained by a minimal tail cut immediately before exsanguination of rats. Blood glucose concentration (mg/dl) was determined with a Glucometer Elite (Model 3901 M).

2.3. Myeloperoxidase assay

Myeloperoxidase tissue activity was determined in homogenates of gastric mucosa with a spectrophotometric assay using the method of Grisham et al. (1986). Briefly, 100 mg of gastric mucosa was homogenized in 1.6 ml of ice-cold 0.02 M EDTA (pH = 4.7) for 60 s. The homogenate was centrifuged at 20 000 rpm for 15 min at 4°C and the supernatant, which contained $< 5\%$ total myeloperoxidase activity was discarded. The pellet was then rehomogenized in an equivalent volume of 0.05 M potassium phosphate buffer (pH = 5.4) containing 0.5% hexadecyltrimethylammonium bromide (HETAB) and 0.01 M EDTA. Aliquots of the supernatant were added to a solution containing 0.5 M sodium-phosphate buffer (pH = 7), 0.3 mM H_2O_2 and 1% *o*-dianisidin. Myeloperoxidase activity was assessed by measuring the H_2O_2-dependent oxidation of *o*-dianisidin. One unit of enzyme activity was defined as the amount of myeloperoxidase that caused a change in absorbance of 1.0/min. at 410 nm and 37°C.

2.4. Assessment of neutrophil infiltration

After evaluation of the ulcer index, stomachs were fixed in formaldehyde for 72 h. The samples of the corpus were dehydrated through a graded series of alcohols and embedded in paraffin. The sections (4 μm thick) were deparaffinized, rehydrated via xylene, mounted on glass slides and stained by hematoxylin and eosine. Slides were examined by an experienced histologist blinded to the treatment regimen. Neutrophil infiltration to the gastric mucosa was determined by counting the numbers of neutrophils on 10

subsequent high power fields (40× objective) in each section in the gastric mucosa under a light microscope (BH12 Olympus).

2.5. Experiments on isolated gastric vessels

Rats (250–300 g) were killed by cervical dislocation and the stomachs with adhering tissue were excised and immediately submerged in cold Krebs–Ringer bicarbonate solution of the following composition (mM): NaCl 135, KCl 5, $NaHCO_3$ 20, glucose 10, $CaCl_2$ 2.5, $MgSO_4 \cdot 7H_2O$ 1.3, KH_2PO_4 1.2, EDTA 0.026. The left gastric artery (lumen diameter at 100 mm Hg = 500 μm) and a small side branch (lumen diameter at 100 mm Hg = 300 μm) on the surface of the stomach were dissected under the microscope. Ring segments of the vessels were mounted in an automated dual small vessel myograph (model 500A, J.P. Trading, Aarhus, Denmark) using 40 μm diameter stainless steel wires. After mounting, the vessels were allowed to equlibrate for 30 min in the Krebs–Ringer bicarbonate solution, which was continuously gassed with 95% O_2–5% CO_2 at 37°C. Thereafter, the relationship between passive wall tension and internal circumference was determined for each vessel by an automated procedure. These characteristics allowed the internal circumference of the vessels to be set at a normalized value, L_0, corresponding to 90% of the internal circumference the vessel would have under a passive transmural pressure of 100 mm Hg, in order to obtain optimal conditions for active force development (Mulvany and Halpern, 1977). After a second equilibration period of at least half an hour, the vessels were contracted three times with Krebs–Ringer bicarbonate solution containing 10^{-5} M noradrenaline and 120 mM K^+ (prepared by appropriate equimolar substitution of NaCl by KCl) to assess contractility of the vessels.

Experiments were performed to evaluate the responsiveness of the main left gastric artery and its side branch to cromakalim and diazoxide. Firstly, a concentration–response curve was made with noradrenaline (10^{-9}–10^{-4} M). After washing, the preparations were precontracted with 10^{-6} M noradrenaline for eliciting a submaximal contraction. On the stabilized precontracted preparations, the relaxing effects of cumulative concentrations of cromakalim (10^{-9}–10^{-5} M) and diazoxide (10^{-9}–10^{-4} M) were assessed sequentially. The concentration–response curves to cromakalim and diazoxide were assessed in changing order. At the end of experiment, the relaxation response to sodium nitroprusside (10^{-9}–10^{-4} M) was also tested. In some experiments, we investigated the influence of L^G-nitro-L-arginine (L-NA, 10^{-4} M), a NO synthase inhibitor, indomethacin (10^{-5} M), a prostaglandin synthase inhibitor and glibenclamide (10^{-5} M), a K_{ATP} channel blocker, on cromakalim and diazoxide induced responses. Therefore, the concentration–response curves of cromakalim and diazoxide were recorded in the presence of these inhibitors.

2.6. Blood pressure measurements

Mean arterial blood pressure was measured in conscious rats by the tail-cuff method before and at 15, 30, 60, and 120 min after i.p. administration of cromakalim (0.1 and 0.3 mg/kg), diazoxide (10 and 30 mg/kg), glibenclamide (10 mg/kg) or their vehicles in indomethacin-treated animals. Rats were prewarmed at 50°C for 3–5 min and gently placed in a restraining cage on a heating plate (37°C). The animals were allowed to adapt for 10–15 min before the measurements.

2.7. Gastric mocosal blood flow measurements

Rats (~200 g) were anaesthetized with phenobarbital (250 mg/kg) and instrumented for measuring mean arterial blood pressure from a carotid artery and recording gastric mucosal blood flow with the hydrogen gas clearance technique (Lippe and Holzer, 1992). This technique yields mean values of blood flow (averaged over a period of 15 min) that are taken every 30 min. Since the stomach was continuously perfused with saline, the drugs (cromakalim 0.1 or 0.3 mg/kg, indomethacin 30 mg/kg) were given intraperitoneally.

After completion of surgery, the rats were allowed to equilibrate for 30 min, after which baseline mean arterial blood pressure and gastric mucosal blood flow were recorded at two time points spaced 30 min apart (−60 min and −30 min in the graph). Thereafter, one group of rats was treated with the vehicle for cromakalim, while the other group received cromakalim (0.1 or 0.3 mg/kg). Thirty minutes after vehicle or cromakalim injection, both groups of rats were injected with indomethacin, and mean arterial blood pressure and gastric mucosal blood flow recorded for the succeeding period of 150 min.

2.8. Drugs

All drugs used were purchased from Sigma (St. Louis, MO) except, misoprostol (Searle) and cimetidine (SmithKline and French Labs.). Indomethacin was dissolved in 5% (w/v) $NaHCO_3$, diazoxide in 1 M NaOH and 0.9% NaCl, glibenclamide in 4% glucose and 0.01 N NaOH, cromakalim in dimethylsulfoxide, atropine in 0.9% NaCl. Cimetidine was suspended in 0.5% methyl cellulose solution. Dilutions were made freshly by saline or Krebs solution. Vehicles were found to have no effect on all the parameters tested.

2.9. Statistical analysis

Values are given as means ± S.E.M. In tension measurements on isolated gastric arteries the maximal responses to relaxant agents (E_{max}) are expressed as the percent decreases of the precontractions. The sensitivities of gastric arteries to relaxant agents are expressed as the effective concentrations that elicited 50% of the maximal response (EC_{50}) and were calculated separately for each

concentration–response curve by probit analysis. EC_{50} values are expressed as negative log *M*. In all experiments, *n* is the number of animals studied. Statistical analyses were performed by using One-way Analysis of Variance (ANOVA) followed by a Tukey–Kramer multiple comparisons test or Student's paired and unpaired *t*-tests where appropriate. For all statistical tests, a *P* value less than 0.05 was considered significant.

3. Results

3.1. Gastric lesions

Six hours after oral administration of indomethacin (30 mg/kg), macroscopically visible gastric mucosal hemorrhagic lesions appeared in the glandular stomach along the mucosal folds (43.4 ± 1.93 mm, $n = 36$). The K^+ channel openers cromakalim (0.3 mg/kg) and diazoxide

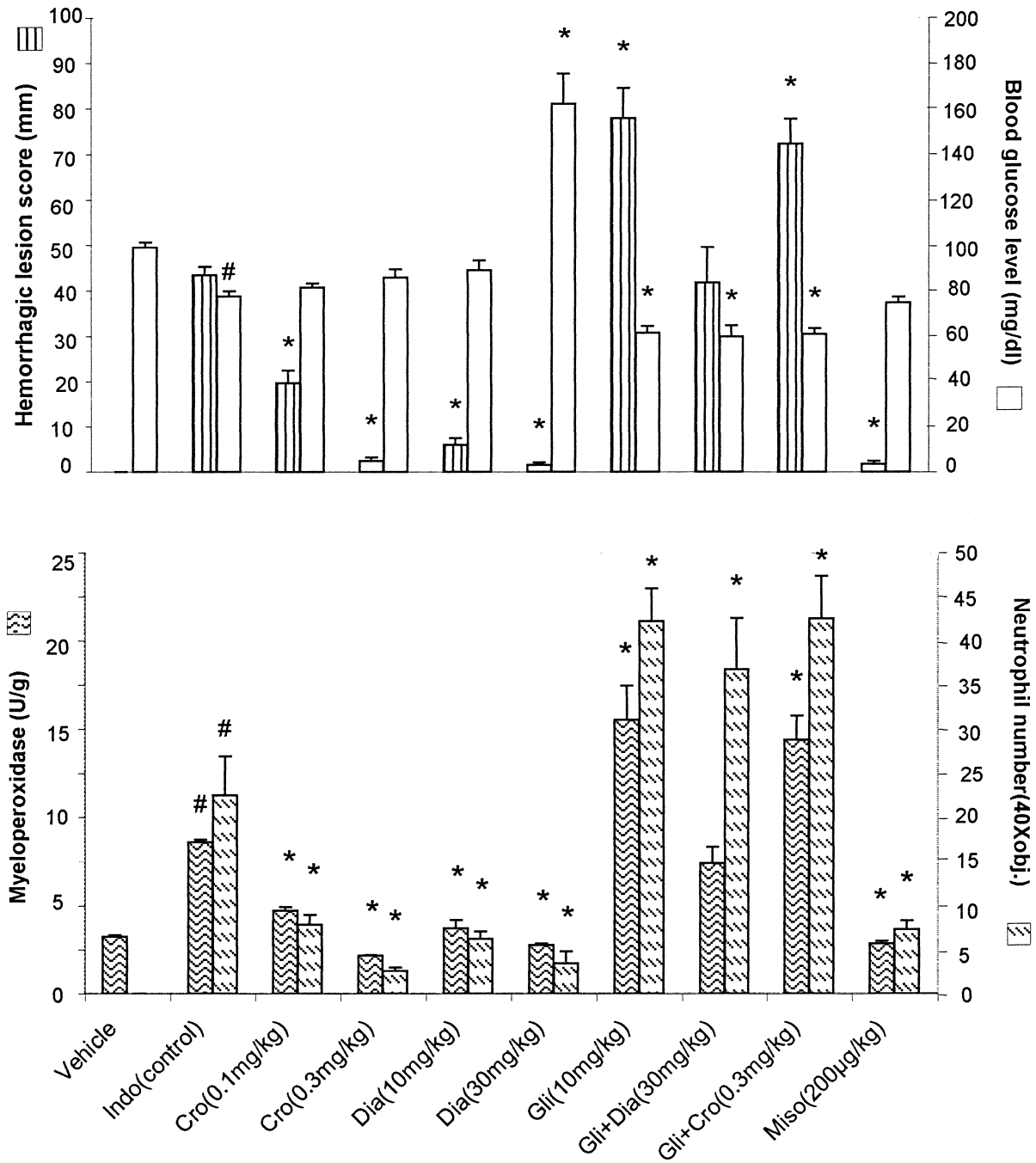

Fig. 1. Effects of cromakalim (Cro, 0.1 and 0.3 mg/kg), diazoxide (Dia, 10 and 30 mg/kg), glibenclamide (Gli, 10 mg/kg) and misoprostol (Miso, 200 μg/kg) on gastric hemorrhagic lesion score (vertical lined bars), blood glucose level (open bars), myeloperoxidase (MPO) activity (hatched bars) and number of neutrophils (hatched bars) in rats treated with indomethacin (Indo). Values are means ± S.E.M.; $n = 36$ for indomethacin group and $n = 6$–16 for other drug treatments in lesion score; $n = 4$–9 in blood glucose level; $n = 4$–6 in myeloperoxidase activity and $n = 5$–8 in neutrophil infiltration. * $P < 0.05$ when compared with indomethacin; # $P < 0.05$ between vehicle and indomethacin group.

(30 mg/kg) completely abolished the mucosal damage induced by indomethacin (2.4 ± 0.76 mm, $n = 16$ and 1.48 ± 0.58 mm, $n = 16$, respectively, $P < 0.05$ vs. indomethacin). Lower doses of cromakalim (0.1 mg/kg) and diazoxide (10 mg/kg) also markedly reduced the lesion score (19.6 ± 2.75 mm, $n = 10$ and 5.9 ± 1.55 mm, $n = 10$, respectively). In contrast, glibenclamide (10 mg/kg) increased the lesions induced by indomethacin (77.8 ± 6.66 mm, $n = 15$, $P < 0.05$ vs. indomethacin). Besides that, preadministration of glibenclamide to the rats given cromakalim (0.3 mg/kg) and diazoxide (30 mg/kg) prevented the gastroprotective effects of these agents (72.3 ± 5.45 mm, $n = 7$ and 41.9 ± 7.85 mm, $n = 10$, respectively). On the other hand, indomethacin-induced lesions were almost completely prevented by misoprostol, cimetidine and atropine (1.6 ± 0.74 mm, $n = 6$; 1.02 ± 0.65 mm, $n = 6$; 3.43 ± 2.12 mm, $n = 6$, respectively, $P < 0.05$ vs. indomethacin) (Fig. 1).

3.2. Blood glucose levels

Blood glucose levels were lower in the animals given indomethacin as compared with those given vehicle only (77.44 ± 2.15 mg/dl, $n = 9$, vs. 98.87 ± 2.49 mg/dl, $n = 8$, $P < 0.05$). Pretreatment with cromakalim (0.1 and 0.3 mg/kg) did not affect (81.2 ± 2.01 mg/dl, $n = 5$, and 85.7 ± 3.56 mg/dl, $n = 7$, respectively) whereas diazoxide at high doses (30 mg/kg) significantly increased and glibenclamide (10 mg/kg) significantly decreased the blood glucose levels in indomethacin-treated rats (162.0 ± 13.52 mg/dl, $n = 5$ and 61.4 ± 2.87 mg/dl, $n = 5$, respectively, $P < 0.05$ vs. 77.44 ± 2.15 mg/dl). Other treatments did not modify the blood glucose level (Fig. 1).

3.3. Myeloperoxidase activity

Indomethacin administration significantly increased myeloperoxidase activity in the gastric mucosa when compared with the corresponding values in control rats (8.60 ± 0.15 U/g, $n = 6$ vs. 3.24 ± 0.10 U/g, $n = 4$, $P < 0.05$). Cromakalim (0.1 and 0.3 mg/kg) and diazoxide (10 and 30 mg/kg) dose-dependently decreased the rise of myeloperoxidase activity caused by indomethacin, bringing it back to control levels (for cromakalim; 4.71 ± 0.22 U/g, and 2.18 ± 0.04 U/g, $n = 4$–6, respectively; for diazoxide: 3.73 ± 0.47 U/g and 2.76 ± 0.11 U/g, $n = 4$–6, respectively). Glibenclamide further increased myeloperoxidase levels (15.54 ± 1.91 U/g, $n = 5$, $P < 0.05$ vs. indomethacin) and also prevented the effects of the K^+ channel openers on myeloperoxidase activity (Fig. 1). Misoprostol (200 μg/kg) decreased myeloperoxidase activity to a similar level as cromakalim and diazoxide (2.85 ± 0.18 U/g, $n = 4$).

3.4. Neutrophil infiltration

Indomethacin (30 mg/kg) caused neutrophil infiltration into the gastric mucosa. Both cromakalim (0.1 and 10 mg/kg) and diazoxide (10 and 30 mg/kg) significantly reduced, whereas glibenclamide increased the number of neutrophils infiltrating the gastric mucosa in indomethacin-treated rats. In addition, glibenclamide reversed the decrease in neutrophil infiltration by cromakalim and diazoxide. Treatment with misoprostol (200 μg/kg) resulted in a similar significant reduction of neutrophil infiltration. Neutrophils were not observed in the gastric mucosa of untreated rats (Fig. 1).

3.5. Gastric arteries

Increasing concentrations of cromakalim (10^{-9}–10^{-5} M) and diazoxide (10^{-9}–10^{-4} M) elicited concentration-related relaxations of the main left gastric artery and its side branch contracted submaximally with noradrenaline (Fig. 2). Cromakalim and diazoxide were similarly effective in the two types of vessels (E_{max} values for cro-

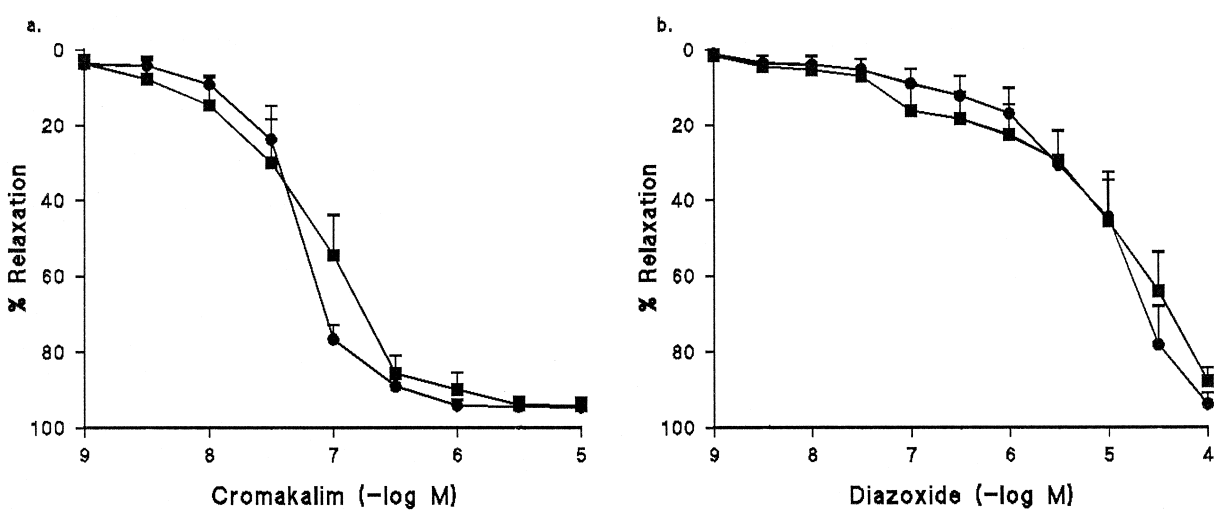

Fig. 2. Concentration–response curves for the effect of cromakalim (a) and diazoxide (b) to relax noradrenaline-contracted rat main left gastric artery (●) and its side branch (■). Values are means ± S.E.M., $n = 6$–8.

makalim: 94.59 ± 1.58%, $n = 6$ and 94.19 ± 2.13%, $n = 8$ in the left main and side branch arteries, respectively). In addition, cromakalim displayed a higher potency than diazoxide in both arteries (Fig. 2 and Table 1). sodium nitroprusside also elicited a strong relaxation of these preparations (Table 1). Glibenclamide (10^{-5} M) almost completely prevented the relaxing effects of cromakalim and diazoxide whereas indomethacin and L-NA did not affect the responses to these agents in both of the arteries (data not shown).

3.6. Arterial blood pressure

Mean arterial blood pressure (mm Hg) in indomethacin-treated conscious rats fell by 19.1 ± 3.1 and 41.2 ± 3.20 mm Hg after injection of cromakalim at 0.1 and 0.3 mg/kg ($n = 4$), respectively. Diazoxide decreased mean arterial blood pressure by 19.35 ± 2.4 and 45.2 ± 3.80 mm Hg at doses of 10 and 30 mg/kg ($n = 4$), respectively. On the other hand, glibenclamide (10 mg/kg) alone did not affect blood pressure of conscious rats (5.2 ± 2.6 mm Hg, $n = 4$) and reversed the reduction of mean arterial blood pressure induced by both cromakalim and diazoxide back to the baseline levels (data not shown).

3.7. Gastric mucosal blood flow

In phenobarbital-anaesthetized rats, cromakalim caused a pronounced fall of mean arterial blood pressure at 0.3 mg/kg but was less hypotensive at 0.1 mg/kg, which persisted during the whole experiment. Gastric mucosal blood flow, however, was not changed by cromakalim and the values of gastric mucosal blood flow over time were very similar in the vehicle/indomethacin and in the cromakalim/indomethacin group. When gastric mucosal vascular conductance (defined as gastric mucosal blood flow/mean arterial blood pressure) was calculated, it turned out that gastric mucosal vascular conductance stayed constant in the vehicle/indomethacin group over the whole duration of the experiment, whereas in the cromakalim/indomethacin group gastric mucosal vascular conductance increased sharply after administration of cromakalim at either dose and stayed elevated for the rest of the experiment (Fig. 3).

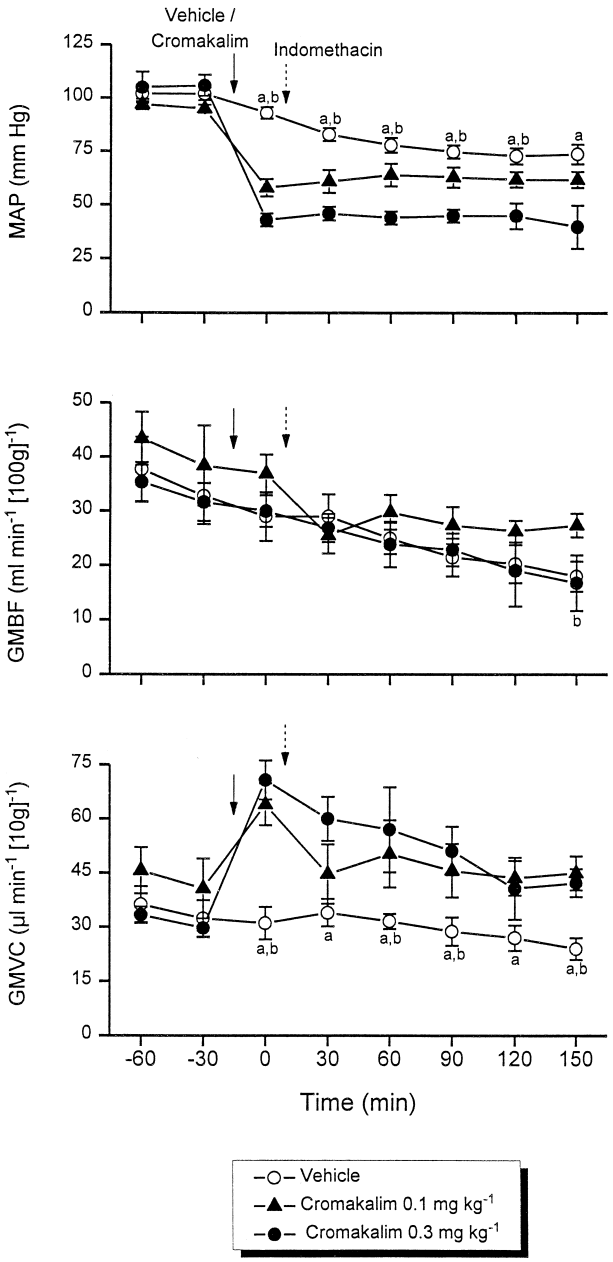

Fig. 3. Effects of vehicle, cromakalim (0.1 and 0.3 mg/kg) and indomethacin (30 mg/kg) injected intraperitoneally at the times indicated, on mean arterial blood pressure (MAP), gastric mucosal blood flow (GMBF) and gastric mucosal vascular conductance (GMVC, calculated as gastric mucosal blood flow divided by mean arterial blood pressure). Values are means ± S.E.M.; $n = 6$; (a) $P < 0.05$ vs. 0.3 mg/kg cromakalim; (b) $P < 0.05$ vs. 0.1 mg/kg cromakalim.

Table 1
E_{max} (maximal relaxations) and EC_{50} values of cromakalim, diazoxide and sodium nitroprusside in noradrenaline pre-contracted rings of the main left gastric artery and its side branch

	n	Main gastric artery		n	Side branch	
		EC_{50}	E_{max}		EC_{50}	E_{max}
Cromakalim	6	7.28 ± 0.08	94.59 ± 1.58	8	7.25 ± 0.19	94.19 ± 2.13
Diazoxide	7	5.03 ± 0.18[a]	93.86 ± 2.99	6	5.11 ± 0.31[a]	87.67 ± 3.40
Sodium nitroprusside	5	7.76 ± 0.16	89.40 ± 1.96	4	7.85 ± 0.18	95.50 ± 1.26

[a] $P < 0.05$ when compared with cromakalim.

4. Discussion

The present study shows that, cromakalim and diazoxide have potent protective effects against indomethacin-

induced gastric hemorrhagic lesions in rats. Their gastroprotective effects are reversed by glibenclamide. Additionally, glibenclamide significantly worsened indomethacin induced gastric injury. Cromakalim and diazoxide are known as openers of K_{ATP} channels (Quast and Cook, 1989; Quast, 1993; Quayle et al., 1995). The ability of glibenclamide to inhibit the response to these agents has generally been used as evidence for the involvement of activation of K_{ATP} channels (Standen et al., 1989; Quayle et al., 1995). It thus seems that the gastroprotective effects of cromakalim and diazoxide are the consequence of K_{ATP} channel opening.

To validate our experimental conditions we used high doses of cimetidine (100 mg/kg) and atropine (10 mg/kg) which have repeatedly been tested in indomethacin-induced gastric injury (Takeuchi et al., 1991, 1997; Ueki et al., 1988). Both antisecretory agents exhibited comparable gastroprotection in our experimental condition. Additionally, misoprostol (200 μg/kg), which is used to prevent the acute gastropathy associated with NSAIDs, also effectively inhibited indomethacin-induced gastric injury. However, misoprostol can have adverse reactions such as diarrhea which may lead to discontinuation of treatment (Walt, 1992). The present study shows that, cromakalim and diazoxide might also be useful in gastroprotection if they have a safe profile at gastroprotective doses.

Gastric injury is the most common adverse reaction of NSAIDs, limiting their use (Fried et al., 1991). The mechanisms responsible for this deleterious action are not completely clear, but impaired mucosal blood flow may be one crucial event. An adequate microvascular blood flow is ensured by endothelial factors such as prostacyclin and NO, which act in concert in the maintenance of mucosal blood flow (Whittle and Lopez-Belmonte, 1993; Wallace and Granger, 1996). Recently, it has been established that K^+ channels play an important role in the regulation of vascular tone (Quast, 1993; Nelson and Quayle, 1995). In the present study, cromakalim and diazoxide fully reversed noradrenaline-induced contractions of the main left gastric artery and its side branch. Their ability to relax gastric arteries were not confined to only noradrenaline-contracted preparations, because serotonin-induced contractions were also sensitive to both agents (data not shown). Cromakalim exhibited a significantly higher potency than diazoxide in relaxing gastric arteries, which is in line with previous studies (Quast and Cook, 1989; Quayle et al., 1995). The presence of L-NA and indomethacin had no influence on the concentration–response curves whereas glibenclamide almost completely inhibited the responsiveness of the gastric arteries to cromakalim and diazoxide. This suggests that the relaxations were related only to K_{ATP} channel opening. Cromakalim is more potent on gastric arteries than on rat mesenteric arteries, studied under the same experimental conditions (Van De Voorde and Vanheel, 1997). Recently, we reported a similar differentiation for levcromakalim, the trans(−)-enantiomer of cromakalim, which shows a much higher potency and efficacy in the reversal of contractions in the human gastroepiploic artery than in human internal mammary artery (Akar et al., 1997). It has been suggested that the vasodilation is important in the maintenance of gastric integrity because the enhancement of blood flow may prevent the activation of inflammatory factors and may remove irritants (Konturek et al., 1993; Wallace and Granger, 1996). Our findings imply that the effective dilator properties of cromakalim and diazoxide on gastric arteries may contribute to their gastroprotective effects by increasing gastric perfusion.

To correlate these in vitro data with in vivo conditions, we tested the effects of cromakalim (0.1 and 0.3 mg/kg) on gastric mucosal blood flow and gastric mucosal vascular conductance by means of the well-standardized method of hydrogen gas clearance. Cromakalim, at 0.1 and 0.3 mg/kg, did not increase gastric mucosal blood flow in phenobarbital anaesthetized rats because its vasodilator effect as shown by an increase in gastric mucosal vascular conductance was cancelled out by the concomittant fall of mean arterial blood pressure. In this context, it needs to be considered that anaesthetics can modify the effects of vasoactive agents, as the hypertensive effect of N^{ω}-nitro-L-arginine methyl ester (L-NAME) under phenobarbital anaesthesia is significantly smaller than that under urethane anaesthesia (Lippe and Holzer, 1992). In the present study, low doses of cromakalim (0.1 mg/kg) and diazoxide (10 mg/kg) had negligible effects on blood pressure of conscious rats, which more closely reflects our condition in the protection experiments. However, the observation that gastroprotective doses of cromakalim (0.1 and 0.3 mg/kg), failed to increase gastric mucosal blood flow indicates that their gastroprotective effects are due to mechanisms other than a rise of gastric blood flow. Similarly, the protective effects of sodium nitroprusside and acetylcholine against reperfusion injury in the gastric mucosa are not linked to local vasodilation since none of them improves local blood flow in the gastric mucosa (Andrews et al., 1994).

A current hypothesis is that neutrophil adhesion to the microvascular endothelium is a critical event in NSAID-induced gastropathy (Wallace, 1993). Prevention of neutrophil adherence by using monoclonal antibodies or neutropenia causes a reduction of NSAID-induced gastric injury (Wallace et al., 1990, 1991, 1993; Yoshida et al., 1993). Neutrophil adherence to the vascular endothelium is observed within 30 min after exposure to indomethacin as a consequence of increased expression of leukocyte and endothelial adhesion molecules (Appleyard et al., 1996). An increase in plasma level of tumor necrosis factor-alpha (TNF-α) has been proposed as the cause of this expression (Rothlein et al., 1991; Appleyard et al., 1996). Santucci et al. (1995) showed that indomethacin caused a dose-dependent and parallel increase in the extent of the gastric mucosal damage, gastric mucosal myeloperoxidase activity and TNF-α level. Recently, it has been reported that

indomethacin-induced gastric mucosal damage peaked 6 h after administration, in coincidence with the peak TNF-α level and peak neutrophil number in gastric mucosa (Ding et al., 1998). The results of an in vitro study show that neutrophils are able to damage the gastric mucosal surface cells by producing superoxide anions (Kozol et al., 1994). In the present study, we observed a linear correlation between the occurrence of gastric injury and neutrophil infiltration into gastric mucosa and the increase of gastric mucosal myeloperoxidase activity in indomethacin-induced gastric injury. This is in line with previous reports (Santucci et al., 1995; Ding et al., 1998). Recruitment and activation of neutrophils in the gastric mucosa are crucial in indomethacin-induced gastric injury. The main finding of the present study is that these indomethacin-induced events in the gastric mucosa can totally be prevented by the K^+ channel openers cromakalim and diazoxide as well as by the prostaglandin analogue misoprostol. A similar effect has recently been reported for the NO donor sodium nitroprusside and for the methyl-xanthine derivative pentoxifylline which also reduce the severity of gastric damage by inhibiting the extent of polymorphonuclear leucocytes margination (Andrews et al., 1994; Santucci et al., 1994). Since gastric injury is causally linked to adherence and activation of neutrophils, we hypothesize that K^+ channel activators may critically affect neutrophil function.

The results of a previous study show that bimakalim, another K_{ATP} channel opener, inhibits the generation of superoxide anion radicals in opsonized zymosan-activated canine polymorphonuclear leucocytes. It has been suggested that K_{ATP} channels may play a significant role in regulating oxygen-derived free radical production in polymorphonuclear leucocytes-induced tissues injury (Pieper and Gross, 1992). In addition, it has been shown that cromakalim and bimakalim significantly reduce myocardial infarct size in dogs, their beneficial effects being reversed by glibenclamide (Grover et al., 1990; Auchampach and Gross, 1994; Mizumura et al., 1995). The cardioprotective effect of bimakalim in ischemic injury is associated with a significant reduction of myeloperoxidase activity (Auchampach and Gross, 1994; Mizumura et al., 1995). Thus, K_{ATP} channel activation may offer a protective mechanism in injury processes by inhibiting polymorphonuclear leucocytes activation and subsequent superoxide production.

Interestingly, glibenclamide alone worsened indomethacin-induced gastric injury and increased neutrophil infiltration into the gastric mucosa and in parallel increased gastric myeloperoxidase activity. These findings cannot be explained by a direct vascular effect because glibenclamide did not change resting or precontracted gastric arterial tone which is in line with our previous findings on the human gastroepiploic artery (Akar et al., 1997). However, in aortic smooth muscle an amplifying effect has been demonstrated between the vasodilators NO, prostacyclin and K^+ channel openers, which is abolished by glibenclamide (Gambone et al., 1997). Similarly, glibenclamide may abolish the interaction between these natural leukocyte inhibitors in our injury model. On the other hand, glibenclamide may also directly inhibit K^+ channels on neutrophils, which has been reported previously (Pieper and Gross, 1992), or increase the sensitivity of adhesion molecules on the endothelium or neutrophils. In a clinical context it is also important to consider possible and dangerous drug interactions between NSAIDs and sulfonylurea antidiabetic agents in causing gastric ulceration.

It has been reported that glucose prefeeding or infusion reduces indomethacin-induced gastric injury (Takeuchi et al., 1990). Takeuchi et al. (1994) suggested that blood glucose level plays a critical role in the development of gastric lesions by modifying gastric motility. In this study, diazoxide and glibenclamide changed the blood glucose level at the dose that we found to influence gastric injury. However, the concentrations of diazoxide which were necessary to induce gastroprotection were not fully comparable with the dose required to produce hyperglycemia. Diazoxide at the low dose (10 mg/kg) produced nearly complete gastroprotection without changing blood glucose level which is in accordance with another study (Quast and Cook, 1989). Moreover, cromakalim had no hyperglycemic effect. These findings show that the gastroprotective effects of K_{ATP} channel openers are not correlated with changes in blood glucose level. On the other hand, cromakalim and diazoxide, similar to misoprostol, have antisecretory effects at high concentrations which are above their effective doses in indomethacin-induced gastric injury (data not shown).

In conclusion, we have demonstrated that cromakalim and diazoxide reduce gastric mucosal injury induced by indomethacin, whereas glibenclamide aggravates this process. Taking together, our data suggest that K_{ATP} channels may have a critical role in the maintenance of gastric mucosal integrity most probably by regulating neutrophil function. Thus, K^+ channel openers may offer an alternative therapeutic approach in the prevention of NSAID-induced gastric injury.

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