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Ischemic preconditioning, the most effective gastroprotective intervention: involvement of prostaglandins, nitric oxide, adenosine and sensory nerves

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Abstract

Various organs, including heart, kidneys, liver or brain, respond to brief exposures to ischemia with an increased resistance to severe ischemia/reperfusion and this phenomenon is called "preconditioning". No study so far has been undertaken to check whether such short, repeated gastric ischemic episodes protect gastric mucosa against severe damage caused by subsequent prolonged ischemia/reperfusion and, if so, what could be the mechanism of this phenomenon. The ischemic preconditioning was induced by short episodes of gastric ischemia (occlusion of celiac artery from one to five times, for 5 min each) applied 30 min before prolonged (30 min) ischemia followed by 3 h of reperfusion or 30 min before topical application of strong mucosal irritants, such as 100% ethanol, 25% NaCl or 80 mM taurocholate. Exposure to regular 30-min ischemia, followed by 3-h reperfusion, produced numerous severe gastric lesions and significant fall in the gastric blood flow and prostaglandin E_2 generation. Short (5-min) ischemic episodes (1–5 times) by itself failed to cause any gastric lesions, but significantly attenuated those produced by ischemia/reperfusion. This protection was accompanied by a reversal of the fall in the gastric blood flow and prostaglandin E_2 generation and resembled that induced by classic gastric mild irritants. These protective and hyperemic effects of standard preconditioning were significantly attenuated by pretreatment with cyclooxygenase-2 and cyclooxygenase-1 inhibitors, such as indomethacin, Vioxx, resveratrol and nitric oxide (NO)-synthase inhibitor, N^G-nitro-L-arginine (L-NNA). The protective and hyperemic effects of standard preconditioning were restored by addition of 16,16 dm prostaglandin E_2 or L-arginine, a substrate for NO synthase, respectively. Gastroprotective and hyperemic actions of standard ischemic preconditioning were abolished by pretreatment with capsaicin-inactivating sensory nerves, but restored by the administration of exogenous CGRP to capsaicin-treated animals. Gene and protein expression of cyclooxygenase-1, but not cyclooxygenase-2, were detected in intact gastric mucosa and in that exposed to ischemia/reperfusion with or without ischemic preconditioning, whereas cyclooxygenase-2 was overexpressed only in preconditioned mucosa. We conclude that: (1) gastric ischemic preconditioning represents one of the most powerful protective interventions against the mucosal damage induced by severe ischemia/reperfusion as well as by topical mucosal irritants in the stomach; (2) gastric ischemic preconditioning resembles the protective effect of "mild irritants" against the damage by necrotizing substances in the stomach acting via "adaptive cytoprotection" and involves several mediators, such as prostaglandin derived from cyclooxygenase-1 and cyclooxygenase-2, NO originating from NO synthase and sensory nerves that appear to play a key mechanism of gastric ischemic preconditioning.

Keywords: Gastric preconditioning; Ischemia/reperfusion; Gastric blood flow; Prostaglandin; Cyclooxygenase; Nitric oxide (NO); Adenosine; CGRP (Calcitonin gene-related peptide); Adaptive cytoprotection

1. Introduction

Ischemia preconditioning refers to a phenomenon in which a tissue is rendered resistant to the deleterious effect of prolonged severe ischemia by previous exposures to brief vascular occlusions (Parratt, 1994). The protective effects of ischemic preconditioning were first described in the heart by Murry et al. (1986). Since that time, ischemic preconditioning has been shown to reduce the extent of myocardial infarct size, as well as the damage to the brain, liver, kidneys and skeletal muscle induced by subsequent

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exposure to severe ischemia folloved by reperfusion in a variety of species (Mounsey et al., 1992; Ishida et al., 1997; Nilsson et al., 2000; Peralta et al., 2000). However, the mechanism of this organ protection by such ischemic preconditioning has not been fully clarified.

The protective activity of ischemic preconditioning is the best documented phenomenon in the heart, where repeated short episodes of coronary occlusion were shown to prevent lethal injury of the myocardium induced by subsequent long-term and severe ischemia/reperfusion (Lawson and Downey, 1993; Przyklenk et al., 1996). In another report, ischemic preconditioning of rat mesenteric venules led to enhanced bioavailability of nitric oxide (NO) and abolished oxidant production resulting in the decrease in the leukocyte adhesion and emigration through mesentery (Davenpeck et al., 1994; Russell et al., 1996). This indicates that ischemic preconditioning also exists in the gut, possibly preventing the mesenteric microvascular barrier dysfunction and activation of excessive amount of NO in the intestine (Davenpeck et al., 1994; Russell et al., 1996; Tsuruma et al., 1996).

The mechanism of ischemic preconditioning remains unclear, but the adenosine that is produced during the ischemic preconditioning was proposed to act as an initiator of this preconditioning in different organs, such as heart and liver, because of the beneficial effect of adenosine in attenuating the injury caused by severe ischemia/reperfusion and an evidence that protective effects of ischemic preconditioning can be reversed by application of adenosine receptor antagonists (Bouchard and Lamontagne, 1996; Peralta et al., 1998).

The question remains whether similar protective effect of ischemic preconditioning can also be observed in the gastric mucosa subjected to longer ischemia/reperfusion or to strong mucosal irritants and, if so, which mechanism is involved in this preconditioning response. Similar protective effects, so-called adaptive cytoprotection, were originally revealed in the stomach by Robert et al. (1983) and confirmed by our group (Konturek et al., 1982) more than two decades ago by showing the protective action of certain mild irritants, such as 20% ethanol, 5% NaCl or 5 mM taurocholate, against the damage induced by these agents applied intragastrically in high mucosal necrotizing concentrations. This action of mild irritants has been predominantly attributed to the protective effects of endogenous prostaglandin; besides prostaglandin, many other protective factors, such as nonprotein sulfhydryl compounds NO, sensory nerves, calcitonin gene-related peptide (CGRP), have been implicated in this phenomenon (Ko and Cho, 1995, 1999; Mercer et al., 1992; Cho et al., 1994; Brzozowski et al., 1996b). The oldest mediator of gastroprotection, prostaglandin, were found to originate from at least two cyclooxygenases (COX), one constitutive (cyclooxygenase-1) playing a physiological role in mucosal homeostasis and another, inducible (cyclooxygenase-2) isoform that is expressed at a site of inflammation (Eberhart and Dubois, 1995; Engelhardt et al., 1996; Dubois et al., 1998). However, the contribution of either isoform of cyclooxygenases in gastric preconditioning has not been explored.

This study was designed to determine whether ischemic preconditioning exists in the stomach and, if so, to elucidate the contribution of endogenous prostaglandin, NO and sensory nerves to gastroprotection against ischemia/reperfusion induced by standard ischemic preconditioning. We also attempted to explore the involvement of adenosine in the mechanism of ischemic preconditioning and to assess the mucosal gene and protein expression of cyclooxygenase-1 and cyclooxygenase-2 in gastric mucosa subjected to ischemic preconditioning with or without prolonged ischemia/reperfusion.

2. Material and methods

Male Wistar rats weighing 180–220 g were used in all studies. Rats were fasted 18 h before the experiment, but they had free access to the drinking water.

2.1. Production of gastric lesions induced by ischemia/reperfusion

Ischemia/reperfusion-induced erosions were produced in 120 rats by the method originally proposed by Wada et al. (1996). Briefly, under pentobarbital anesthesia (50 mg/kg, i.p.), the abdomen was opened, the celiac artery identified and clamped with a small device for 30 min followed by removal of the clamp to obtain reperfusion. In addition, short ischemia (occlusion of celiac artery 1–5 times for 5-min ischemic preconditioning) was applied 30 min before subsequent exposure to longer (regular) 30 min of ischemia (also induced by clamping of celiac artery) and followed by 3 h of reperfusion. Supplementation dose of the anesthetic was given i.p. to the rats to maintain at least 3 h anesthesia. The respective control group included the sham-operated control animals, whose the celiac artery was only slightly manipulated, but not occluded. First, we attempted to determine the effect of various time periods of gastric ischemic preconditioning on the lesions induced by regular ischemia/reperfusion. For this purpose, rats were preconditioned with single episode of gastric preconditioning ranging from 37 up to 300 s before the exposure to 30 min of ischemia followed by 3 h of reperfusion. Second, we wanted to know whether the increasing number of short ischemic episodes affects the lesions induced by ischemia/reperfusion. For this purpose, gastric mucosa was pretreated with 1–5 episodes of short ischemia (5 min each) before the exposure to regular ischemia/reperfusion. In another group of rats, the duration of protective effect of standard ischemic preconditioning (2×5 min occlusion)

against the gastric erosions caused by regular ischemia/reperfusion was studied. The duration of the preconditioning effect was examined in rats pretreated with standard (2×5 min occlusion) ischemic preconditioning followed 1, 2, 4, 8 or 12 h later by regular ischemia/reperfusion.

2.2. Effect of suppression of cyclooxygenase-1 and cyclooxygenase-2 activity on gastric ischemic preconditioning

In separate group of rats, the pretreatment with cyclooxygenase inhibitors was employed 30 min prior to gastric preconditioning followed by 3 h of ischemia/reperfusion in order to determine whether suppression by nonselective or selective inhibitors of cyclooxygenase-1 and cyclooxygenase-2 influences the protective action of the preconditioning.

Several groups of rats, each consisting of 6–8 animals, were given 30 min before gastric preconditioning one of the following treatments: (1) vehicle (saline); (2) resveratrol (1,3-Benzenediol,5-[2-(4-hydroxyphenyl) ethenyl], 10 mg/kg, i.g.), a selective cyclooxygenase-1 inhibitor (Jang et al., 1997); (3) Vioxx (5 mg/kg, i.g.), the highly selective cyclooxygenase-2 inhibitor (Brzozowski et al., 1999); and (4) indomethacin (5 mg/kg, i.p.), a nonselective cyclooxygenase inhibitor (Wallace et al., 1998). At the dose used in present study, indomethacin has been shown previously to inhibit gastric prostaglandin E_2 generation capability by $\sim$90% without causing by itself any mucosal damage (Konturek et al., 1998). The dose of Vioxx was selected on the basis of previous studies, showing that this agent failed to affect the generation of prostaglandin E_2 in intact gastric mucosa, but inhibited significantly the gastric prostaglandin E_2 production in ulcerated gastric mucosa (Brzozowski et al., 1999). Resveratrol (Cayman Chemical, Ann Arbor, MI, USA) was first dissolved in absolute ethanol to obtain the stock solution of 50 mg/ml and then diluted to the desired concentration with the isotonic saline. Vioxx (Merck Sharp & Dohme) was first dissolved in methanol to obtain the stock solution 50 mg/ml and then diluted to the desired concentration with isotonic saline as described previously (Brzozowski et al., 1999). Resveratrol and Vioxx were used in a dose (10 and 5 mg/kg, i.g., respectively) that were shown by our group to inhibit the prostaglandin E_2 generation in the gastric mucosa injured by ischemia/reperfusion (Brzozowski et al., 1999). Control rats received the corresponding vehicle. Our preliminary studies (data not shown) confirmed that none of the cyclooxygenase inhibitors used in this study produced by itself any gastric lesions at the doses tested.

In another group of animals subjected to standard ischemic preconditioning and then to ischemia/reperfusion with or without treatment with cyclooxygenase-1 and cyclooxygenase-2 inhibitors, the prostaglandin were replaced using 16,16 dimethyl prostaglandin E_2 (Upjohn, Kalamazoo, MI, USA) applied in a dose of 1 μg/kg, i.g. that was found in our preliminary study to be without any influence on gastric lesions caused by ischemia/reperfusion and accompanying fall in gastric blood flow (data not shown). For this purpose, 16,16 dimethyl prostaglandin E_2 analog was administered together with each cyclooxygenase-1 or cyclooxygenase-2 inhibitor starting 30 min prior to standard ischemic preconditioning followed by 3 h of ischemia/reperfusion.

The area of gastric lesions was determined using a planimeter (Morphomat, Carl Zeiss, Berlin, Germany) under blinded conditions according to the method described previously (Konturek et al., 1992).

2.3. Involvement of NO in the protective effect of gastric preconditioning

The implication of NO in the effect of gastric preconditioning on damage induced by ischemia/reperfusion was determined by three ways: (1) by using N^G-nitro-L-arginine (L-NNA) applied i.g. in a dose of 20 mg/kg to suppress nonspecifically the activity of nitric oxide (NO)-synthase (NOS) (Whittle et al., 1990); (2) by indirect measurement of NOS product, i.e. NO in gastric lumen (Whittle, 1994); and (3) by addition to L-NNA of L-arginine, a substrate for NOS or D-arginine, which is not a substrate for NO (Brzozowski et al., 1997a). The rats with gastric lesions induced by regular ischemia/reperfusion were pretreated either with: (1) sham operation or standard ischemic preconditioning (occlusion of celiac artery twice for 5 min) alone; (2) L-NNA (20 mg/kg, i.g.) with or without the preconditioning; (3) L-arginine (200 mg/kg, i.g.) plus L-NNA (20 mg/kg, i.g.) combined with the preconditioning; and, finally, (4) D-arginine (200 mg/kg, i.g.) plus L-NNA (20 mg/kg, i.g.) combined with the preconditioning.

The luminal concentration of NO was quantified indirectly as nitrate (NO_3^-) and nitrite (NO_2^-) levels in the gastric contents using the nitrate/nitrite kit purchased from Cayman Lab (Michigan, USA) as described in details before (Brzozowski et al., 1997b). This method is based on the Griess reaction and generation of chromophore absorbing at 595 nm, according to the original procedure reported previously (Green et al., 1981). Since NO released by epithelial cells into the gastric lumen is quickly transformed into NO_3^- and NO_2^- (Whittle, 1994), we measured photometrically the sum of both these products of NOS as an index of production of NO by the enzyme in the gastric mucosa. For this purpose, the gastric content was aspirated just before the removal of the stomach following the i.g. injection of 1 ml of saline to wash out the luminal content. After centrifugation for 10 min at 3000 rpm, the samples were mixed with Griess reagent from the commercially available kit. In all tests, including gastric preconditioning with or without the combination with L-NNA and L-arginine or D-arginine, the gastric blood flow was measured in the

oxyntic mucosa in each group of animals in similar manner as mentioned before and expressed as the percent control value recorded in vehicle-treated gastric mucosa.

2.4. Implication of sensory afferent nerves and CGRP in gastric ischemic preconditioning

In tests with involvement of sensory nerves and neuropeptides in gastroprotection induced by gastric preconditioning, rats with capsaicin-induced deactivation of these nerves or those pretreated with calcitonin gene-related peptide (CGRP) receptor antagonist, CGRP-(8–37), respectively (Brzozowski et al., 1999; Ichikawa et al., 2000; Tubaro et al., 2000), were used. The role of sensory afferent nerves in protective effects of ischemic preconditioning was tested in rats with capsaicin-induced deactivation of these nerves. For this purpose the animals were pretreated with capsaicin (Sigma, St. Louis, MO) injected s.c. for 3 consecutive days at a dose of 25, 50 and 50 mg/kg about 2 weeks before the experiment as described previously (Takeuchi et al., 1994). All injections of capsaicin were performed under ether anesthesia to counter the pain reactions and respiratory impairment associated with injection of this agent. To check the effectiveness of the capsaicin denervation, a drop of 0.1 mg/ml solution of capsaicin was instilled into the eye of each rat and the protective movements were counted as described previously (Brzozowski et al., 1996b). Control rats received injections with vehicle (saline). All animals pretreated with capsaicin showed negative wiping movement test, thus confirming functional denervation of the capsaicin sensitive nerves. Standard ischemic preconditioning was induced in rats with intact or capsaicin-deactivated nerves and this was followed by ischemia/reperfusion, according to the procedure described above. In some group of capsaicin denervated rats, CGRP (10 μg/kg) was applied s.c. 30 min before standard preconditioning followed by exposure to ischemia/reperfusion, in order to check, whether deficiency of endogenous CGRP due to functional ablation of sensory nerves could be abolished by administration of exogenous neuropeptide at a dose that was reported to reverse the effects of capsaicin deactivation on gastric mucosa (Brzozowski et al., 1996b). The CGRP receptor antagonist CGRP-(8–37) was applied 30 min before standard ischemic preconditioning or vehicle followed 30 min later by ischemia/reperfusion. The experimental protocol included the following study groups, each consisting of 6–8 animals: (1) vehicle (saline) followed 30 min later by standard preconditioning in rats with intact afferent nerves; (2) standard preconditioning followed 30 min later by ischemia/reperfusion in rats with intact sensory nerves; (3) vehicle (saline) followed 30 min later by ischemia/reperfusion in rats with capsaicin-deactivated afferent nerves or those pretreated with CGRP-(8–37) (100 μg/kg, i.p.); (4) standard preconditioning followed 30 min later by ischemia/reperfusion in rats with capsaicin-deactivated afferent nerves or those pretreated with CGRP-(8–37) (100 μg/kg, i.p.).

In this series of experiments, the area of lesions and the gastric blood flow were measured in similar manner as mentioned above.

2.5. Implication of adenosine in ischemic gastric preconditioning

The involvement of adenosine in mediating of the effect of preconditioning on the gastric mucosa was determined by two ways: (1) by pretreatment with 8-*p*-sulphophenyl theophylline at a dose (10 mg/kg, i.g.) that was reported to inhibit adenosine receptors and to attenuate the effect of ischemic preconditioning on the heart infarct size (Hoshida et al., 1994); and (2) by the application of exogenous adenosine (10 mg/kg, i.g.) to check whether pretreatment with exogenous adenosine can protect the gastric mucosa lesions induced by regular ischemia/reperfusion.

2.6. Measurement of gastric blood flow

At the termination of each experiment, the gastric blood flow was measured by H_2-gas clearance technique. If necessary, rats were lightly anesthetized with ether, the abdomen was opened and the stomach was exposed. The gastric blood flow was measured in the oxyntic gland area of the stomach by means of local H_2-gas clearance method using an electrolytic regional blood flow meter (Biomedical Science, Model RBF-2, Japan) as described previously (Brzozowski et al., 1999). The measurements were calculated in three areas of the mucosa and the mean absolute values (ml/100 g min) of these measurements were calculated and expressed as percent changes from those recorded in control animals treated with vehicle.

2.7. Determination of mucosal generation of prostaglandin E_2

In groups of rats exposed to standard ischemic preconditioning followed by regular ischemia/reperfusion without or with pretreatment with COX-inhibitors, the mucosal samples form the oxyntic gland area were taken by biopsy (about 200 mg) from gastric mucosa without mucosal lesions immediately after the animals were sacrificed to determine the mucosal generation of prostaglandin E_2 by radioimmunoassay (RIA) as described previously (Konturek et al., 1998). The mucosal samples were placed in preweighed Eppendorf vial and 1 ml of Tris buffer (50 mM, pH 9.5) was added to each vial. The samples were finely minced (during 15 s) with scissors, washed and centrifuged for 10 s, the pellet being resuspended again in 1 ml of Tris. Then, each sample was incubated on a Vortex mixer for 1 min and centrifuged for 15 s. The pellet was weighed and the supernatant was transferred to a second Eppendorf vial containing indomethacin (10 mM) and kept

at −20 °C until the radioimmunoassay. The capability of the mucosa to generate PGE_2 was expressed in nanograms of wet tissue weight.

2.8. Expression of cyclooxygenase-1 and cyclooxygenase-2 mRNA transcripts in the gastric mucosa determined by reverse transcriptase-polymerase chain reaction (RT-PCR)

Cyclooxygenase-1 and cyclooxygenase-2 mRNA were determined by RT-PCR in the gastric mucosa of intact rats or those exposed to ischemia/reperfusion with or without ischemia preconditioning. Samples of the gastric oxyntic mucosa (about 500 mg) were scraped off on ice using glass slide and then immediately snap frozen in liquid nitrogen and stored at −80 °C. Total RNA was isolated from the gastric oxyntic mucosa, according to Chomczynski and Sacchi (1987), using a rapid guanidinum isothiocyanate/phenol chloroform single step extraction kit from Stratagene (Heidelberg, Germany).

First strand cDNA was synthesized from total cellular RNA (5 μg) using 200 U Strata Script TM reverse transcriptase and oligo (dt) primers (Stratagene). The primers for cyclooxygenase-1 and cyclooxygenase-2 were synthesized by Biometra (Gottingen, Germany). The primer sequences were designed according to the published cDNA sequence for the rat β-actin and cyclooxygenases (Nudel et al., 1983; Xie et al., 1991; Kennedy et al., 1993; Masferrer et al., 1994; Feng et al., 1995). The COX-1 primer sequences were as follows: upstream, 5′-AGC CCC TCA TTC ACC CAT CAT TT; downstream, 5′-CAG GGA CGC CTG TTC TAC GG. The expected length of this PCR product was 561 bp. The COX-2 primer sequences were as follows: upstream, 5′-ACA ACA TTC CTT CCT TC; downstream, 5′-CCT TAT TTC CTT TCA CAC C. The expected length of this PCR product was 201 bp. Concomitantly, amplification of control rat β-actin was performed on the same samples to verify the RNA integrity. DNA amplification was carried out under the following conditions: denaturation at 94 °C for 1 min, annealing at 60 °C for 45 s and extension at 72 °C for 45 s. Each PCR-product (8 μl) was electrophoresed on 1.5% agarose gel stained with ethidium bromide and then visualized under UV light. Location of predicted PCR product was confirmed by using a 100-bp ladder (Gibco BRL/Life Technologies, Eggenstein, Germany) as standard marker.

2.9. Protein extraction and analysis of cyclooxygenase-2 expression in the gastric mucosa by Western Blot

Shock-frozen gastric tissue was homogenized in lysis buffer (100 mmol Tris–HCl, pH = 7.4, 15% glycerol, 2 mmol EDTA, 2% sodium dodecyl sulfate (SDS), 100 mmol D,L-dithiothreitol by the addition of 1:20 dilution of aprotinin and 1:50 dilution of 100 mmol phenylmethylsulfonyl fluoride as described in detail recently (Konturek et al., 1999). Insoluble material was removed by centrifugation at 12,000 × *g* for 15 min. Approximately, 100 μg of cellular protein extract was loaded into a well, separated electrophoretically through a 13.5% SDS-polyacrylamide gel and transferred onto Sequi-Blot TMPVDF membrane (BioRad, USA) by electroblotting. The specific primary rabbit polyclonal antibody against cyclooxygenase-1 and cyclooxygenase-2 (dilution 1:500, Santa Cruz, USA) or rabbit polyclonal β-actin antibody against was added to membrane, followed by an anti-rabbit immunoglobulin G horseradish peroxidase-conjugated secondary antibody (dilution 1:2000, Santa Cruz). Nonisotopic visualization of immuno-complexes was achieved by chemiluminescence using BM Chemiluminescence Blotting Substrate (Boehringer Mannheim, Germany) and the developed membrane was exposed to an X-ray film (Kodak, Wiesbaden, Germany). Comparisons between different treatment groups were made by determining the cyclooxygenase-1- and cyclooxygenase-2/β-actin ratio of the immunoreactive area by densitometry.

2.10. Statistical analysis

Results are expressed as means ± S.E.M. The significance of the difference between means was evaluated using analysis of variance followed by Duncan's test with a level of confidence at $P < 0.05$.

3. Results

3.1. Effect of short ischemic episodes on the gastric lesions induced by ischemia / reperfusion insult and the accompanying changes in the GBF

Fig. 1 shows the effects of various time duration of single short ischemic episode lasting from 37 up to 300 s on gastric lesions and accompanying changes in the gastric

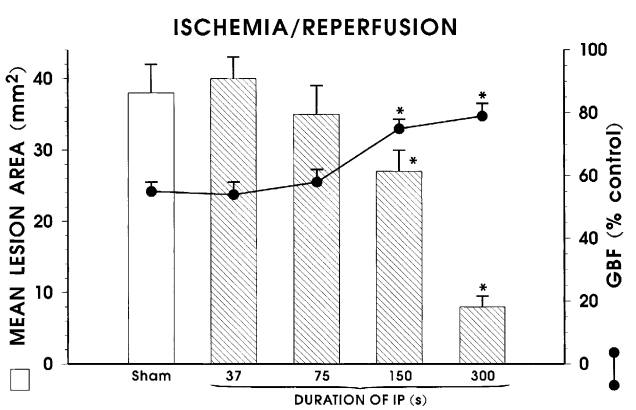

Fig. 1. Mean area of gastric lesions (columns) and gastric blood flow (GBF) (lines) in the gastric mucosa of rats pretreated with sham (control) or gastric preconditioning (IP) lasting from 37 up to 300 s and then exposed to 30 min of ischemia followed by 3 h of reperfusion. Results are mean ± S.E.M. of 6–8 rats. Asterisk indicates a significant change as compared with the value obtained in sham-control animals.

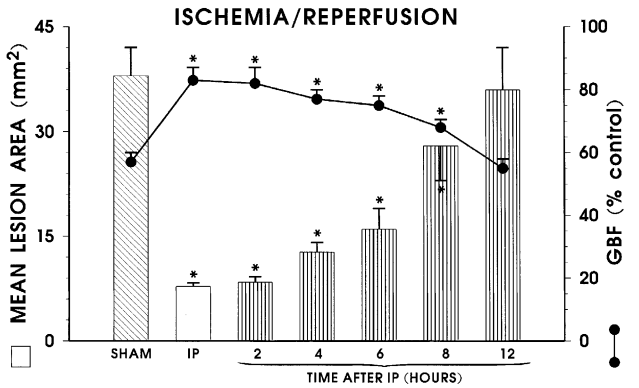

Fig. 2. Effect of standard ischemic preconditioning followed 2, 4, 6, 8 and 12 h later by regular ischemia/reperfusion on the area of gastric lesions (columns) and gastric blood flow (GBF) (lines). Results are mean ± S.E.M. of 6–8 rats. Asterisk indicates a significant change as compared with the value obtained in sham-operated control rats.

blood flow induced by regular ischemia/reperfusion. Ischemic episodes shorter than 75 s failed to influence significantly the area of ischemia/reperfusion-induced gastric lesions and to affect the gastric blood flow. With prolongation of ischemic episodes up to 150 or 300 s applied before regular ischemia/reperfusion, a significant reduction in the area of acute gastric lesions and a significant rise of the gastric blood flow were observed. The short ischemia of 300 s (5 min) that caused reduction in ischemia/reperfusion-induced lesions by about 80% was used as a standard ischemic preconditioning and used in subsequent studies.

In order to select how many episodes of short ischemia are necessary to afford the maximal protective action against the damage evoked by prolonged ischemia/reperfusion, we tested the effect of various numbers of standard (5 min) ischemia ranging from 1 to 5 on the area of gastric erosions induced by regular ischemia/reperfusion. Single standard (5 min) preconditioning episode reduced the area of ischemia/reperfusion erosions by about 67%. Increase in number of standard ischemic episodes to 2 (2×5 min occlusion) did not result in any further significant reduction in lesion area caused by regular ischemia/reperfusion. The gastric blood flow in the intact stomach averaged 53 ± 6 (taken as 100%) and this was significantly reduced (by about 40%) at the end of 3 h of reperfusion that followed 30 min of ischemia. A single standard ischemic episode increased significantly the gastric blood flow by about 25% as compared to that recorded in sham-operated controls exposed to regular ischemia/reperfusion. Exposure of the gastric mucosa to 2–5 ischemic episodes produced similar rise in the gastric blood flow, but this increase was not significantly different than that obtained in animals with single ischemic episode.

Fig. 2 shows the duration of the protective effect of standard (2×5 min) ischemic preconditioning against regular ischemia/reperfusion. This protection followed by the rise in the gastric blood flow was observed at 2 h after the beginning of ischemic preconditioning and found to last up to 8 h. However, after 12 h since the preconditioning, such a protective effect and accompanying hyperemia were lost and the area of gastric lesions reached the value not significantly different from that recorded in rats exposed to regular ischemia/reperfusion.

As shown in Fig. 3, the pretreatment with standard preconditioning resulted in a significant attenuation of gastric lesions induced by regular ischemia/reperfusion

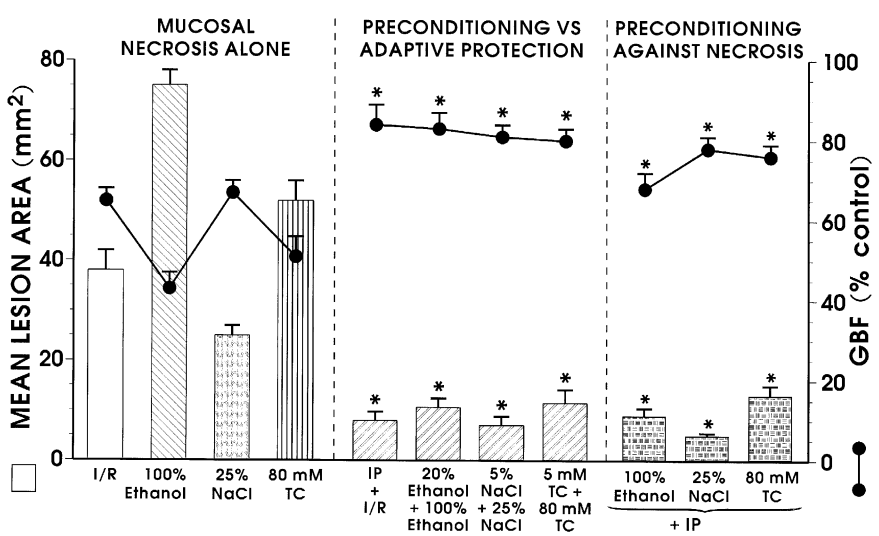

Fig. 3. Effect of the pretreatment with standard ischemic preconditioning (IP), 20% ethanol, 5% NaCl and 5 mM TC on the area of gastric lesions (columns) and accompanying changes in the GBF (lines) induced by the exposure to regular ischemia/reperfusion (I/R), 100% ethanol, 25% NaCl and 80 mM taurocholate (TC). Results are mean ± S.E.M. of 6–8 rats. Asterisk indicates a significant change as compared with the value obtained in gastric mucosa without IP.

and in accompanying rise in the gastric blood flow similar to that presented in Figs. 1 and 2. This gastroprotection and the rise in the gastric blood flow achieved by a standard preconditioning against gastric lesions evoked by regular ischemia/reperfusion were not significantly different from those obtained in animals pretreated with mild irritants, such as 20% ethanol, 5% NaCl and 5 mM taurocholate, against mucosal damage induced by 100% ethanol, 25% NaCl and 80 mM taurocholate, respectively. Standard preconditioning applied 30 min before 100% ethanol, 25% NaCl or 80 mM taurocholate attenuated significantly the gastric lesions induced by each of these strong irritants applied alone (Fig. 3). The gastric blood flow in preconditioned gastric mucosa followed by intragastric application of necrotizing agents rose to similar extent to those treated with this preconditioning and exposed to regular ischemia/reperfusion (Fig. 3).

3.2. Effect of nonselective and selective inhibitors of cyclooxygenase-1 and cyclooxygenase-2 on the protection induced by gastric preconditioning and the changes in the gastric blood flow and prostaglandin E_2 generation in the gastric mucosa

As shown in Fig. 4, standard (2×5 min) preconditioning attenuated the ischemia/reperfusion-induced gastric lesions and raised the GBF to the extent similar as presented in Figs. 2 and 3. Indomethacin, Vioxx and resveratrol significantly enhanced the gastric lesion area induced by ischemia/reperfusion. In rats pretreated with indomethacin, Vioxx or resveratrol and then exposed to standard preconditioning, the area of erosions caused by regular ischemia/reperfusion decreased significantly below that recorded in those with ischemia/reperfusion alone and this effect was accompanied by the significant increase in the gastric blood flow as compared to the respective value obtained in ischemia/reperfusion rats.

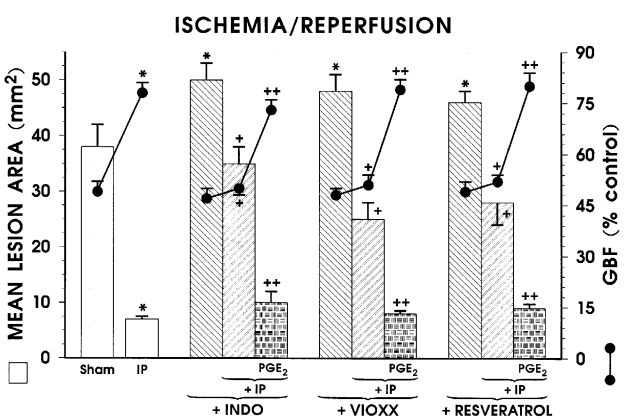

Fig. 4. Effect of standard ischemic preconditioning (IP) with or without pretreatment with indomethacin (5 mg/kg, i.p.), Vioxx (5 mg/kg, i.g.) and resveratrol (10 mg/kg, i.g.) administered with or without addition of 16,16 dm prostaglandin E_2 (PGE_2 1 μg/kg, i.g.) on the area of gastric lesions (columns) and accompanying changes in the GBF (lines) induced by the exposure to regular ischemia/reperfusion (I/R). Results are mean ± S.E.M. of 6–8 rats. Asterisk indicates a significant change as compared with the value obtained in sham-control gastric mucosa. Cross indicates a significant change as compared with the value obtained in rats without treatment with COX inhibitors. Double cross indicates a significant change as compared to the value obtained in rats without PGE_2 administration.

Table 1
Effect of standard ischemic preconditioning (IP) without or with the pretreatment with resveratrol (10 mg/kg, i.g.), Vioxx (5 mg/kg, i.g.) and indomethacin (5 mg/kg, i.p.), on the mucosal generation of PGE_2 in gastric mucosa exposed to regular ischemia/reperfusion
Results are mean ± S.E.M. of 8–10 rats.

Type of test	PGE_2 generation (ng/g)
Intact	128 ± 12
Without ischemia / reperfusion	
IP	154 ± 10[a]
Indomethacin	33 ± 4[a]
Vioxx	118 ± 13
Resveratrol	104 ± 9[a]
With ischemia / reperfusion	
Sham	68 ± 8[a]
IP	118 ± 6[b]
Indomethacin + IP	18 ± 3[c]
Vioxx + IP	64 ± 5[c]
Resveratrol + IP	55 ± 4[c]

[a] Indicates a significant change as compared to the value obtained in intact gastric mucosa.
[b] Indicates a significant change as compared to the value obtained in gastric mucosa exposed to ischemia/reperfusion.
[c] Indicates a significant change as compared to the respective value obtained in gastric mucosa not exposed to ischemia/reperfusion.

The generation of prostaglandin E_2 in the intact gastric mucosa averaged 128 ± 12 ng/g of wet tissue weight (Table 1). The exposure of gastric mucosa to regular ischemia/reperfusion that caused gastric lesions produced a significant decrease (about 50%) in the prostaglandin E_2 generation as compared to the values recorded in the intact gastric mucosa (68 ± 8 vs. 128 ± 12 ng/g of wet tissue weight, Table 1). As described above, the administration of indomethacin (5 mg/kg, i.p.) that suppressed mucosal generation of prostaglandin E_2 by about 90% increased significantly the mean area of ischemia/reperfusion lesions and this effect was accompanied by a significant fall in the gastric blood flow (Fig. 4, Table 1). Vioxx failed to affect significantly the generation of PGE_2 in the gastric mucosa not exposed to ischemia/reperfusion. When Vioxx was applied i.g. in a dose of 5 mg/kg, the significant increase in gastric lesions and the fall in the GBF and PGE_2 were observed. Resveratrol, which also augmented significantly the area of gastric lesions induced by ischemia/reperfusion, produced a significant fall in the prostaglandin E_2 generation (Table 1).

The addition of prostaglandin E_2 (1 μg/kg, i.g.), which by itself failed to influence significantly the ischemia/reperfusion lesions (data not shown), attenuated significantly the enhancement in area of these lesions and accompanying fall in gastric blood flow induced by indomethacin (Fig. 4). Prostaglandin E_2 added to indomethacin, Vioxx or resveratrol abolished completely the increase in area of gastric lesions and accompanying fall in the gastric blood flow induced by administration of these COX-inhibitors (Fig. 4).

3.3. Effect of L-NNA on gastric lesions, gastric blood flow and no production in gastric mucosa exposed to ischemia/reperfusion with or without gastric preconditioning

Fig. 5 shows the results of tests with standard preconditioning with or without addition of L-NNA or the combination of L-NNA plus L-arginine or D-arginine on the area of gastric luminal contents of NO_3^-/NO_2^- and gastric blood flow. The pretreatment with standard preconditioning resulted in usual attenuation of lesion area and an increase in gastric blood flow and produced a significant rise in luminal contents NO_3^-/NO_2^-. L-NNA applied i.g. in a dose of 20 mg/kg, aggravated significantly the lesions induced ischemia/reperfusion and decreased the gastric blood flow and luminal release of NO degradation products as compared to those in vehicle-treated animals. Such treatment with L-NNA abolished the decrease in ischemia/reperfusion lesions, the rise in gastric blood flow and the production of NO into gastric lumen recorded in animals subjected to gastric preconditioning applied before ischemia/reperfusion (Fig. 5). Addition of L-arginine, but not D-arginine, to the combination of L-NNA and short ischemia restored the protective effect, the rise in gastric blood flow and luminal NO_3^-/NO_2^- content to the levels observed in rats pretreated with short ischemia (Fig. 5).

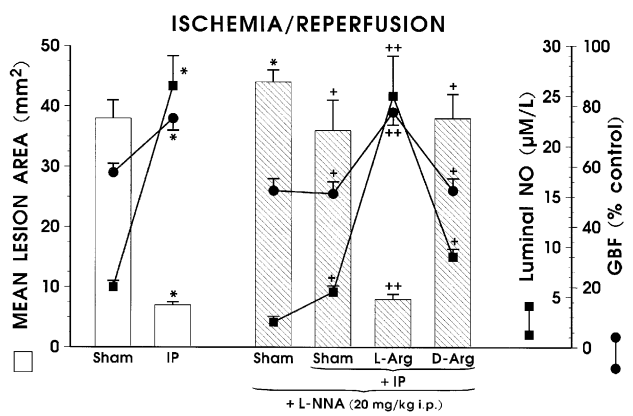

Fig. 5. Effect of standard ischemic preconditioning (IP) with or without pretreatment with N^G-nitro-L-arginine (L-NNA 20 mg/kg, i.p.) applied with or without the combination with L-arginine (L-Arg, 200 mg/kg, i.g.) or D-arginine (D-Arg, 200 mg/kg, i.g.) on the area of gastric lesions (columns) and accompanying changes in the GBF and gastric luminal NO concentration (lines) induced by the exposure to regular ischemia/reperfusion (I/R). Results are mean ± S.E.M. of 6–8 rats. Asterisk indicates a significant change as compared with the value obtained in sham-control gastric mucosa. Cross indicates a significant change as compared with the value obtained in rats without treatment with L-NNA. Double cross indicates a significant change as compared to the value obtained in rats without L-Arg administration.

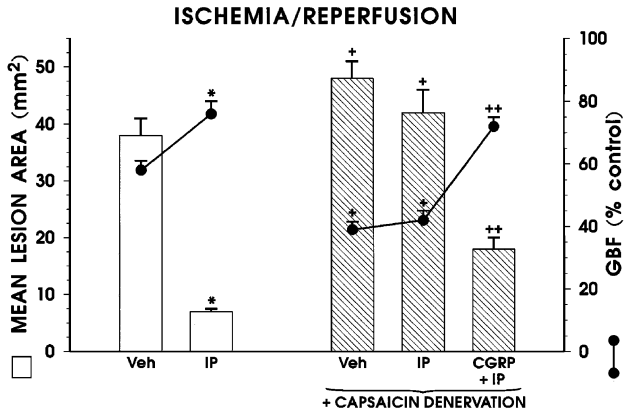

Fig. 6. Effect of standard ischemic preconditioning (IP) in rats with intact or capsaicin-deactivated sensory nerves with or without addition of calcitonin gene related peptide (CGRP, 10 μg/kg, i.p.) on the area of gastric lesions (columns) and accompanying changes in the GBF (lines) induced by the exposure to standard ischemia/reperfusion. Results are mean ± S.E.M. of 6–8 rats. Asterisk indicates a significant change as compared with the value obtained in sham-control gastric mucosa. Cross indicates a significant change as compared with the value obtained in rats without capsaicin denervation. Double cross indicates a significant change as compared with the value obtained in capsaicin-denervated rats.

3.4. Effect of capsaicin denervation and CGRP-(8–37) on gastric lesions induced by regular ischemia/reperfusion and gastric blood flow in rats with or without pretreatment with standard preconditioning

The results of capsaicin deactivation of sensory nerves and the pretreatment with CGRP receptor antagonist,

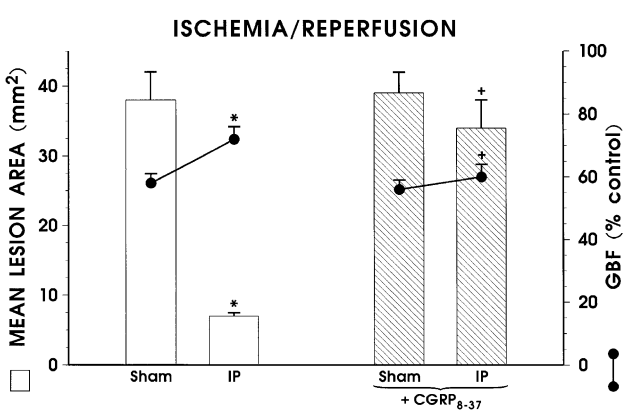

Fig. 7. Effect of standard ischemic preconditioning (IP) with or without the pretreatment with CGRP-(8–37) (100 μg/kg, i.p.) on the area of gastric lesions (columns) and accompanying changes in the GBF (lines) induced by the exposure to standard ischemia/reperfusion. Results are mean ± S.E.M. of 6–8 rats. Asterisk indicates a significant change as compared with the value obtained in sham-control gastric mucosa. Cross indicates a significant change as compared with the value obtained in rats without CGRP-(8–37) pretreatment.

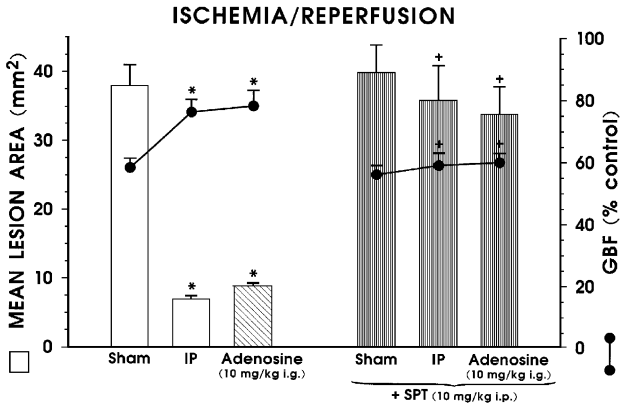

Fig. 8. Effect of standard ischemic preconditioning (IP) and adenosine (10 mg/kg, i.g.) applied alone or combined with 8-*p*-sulphophenyl theophylline (SPT; 10 mg/kg, i.p.) on the area of gastric lesions (columns) and accompanying changes in the GBF (lines) induced by the exposure to standard ischemia/reperfusion. Results are mean ± S.E.M. of 6–8 rats. Asterisk indicates a significant change as compared with the value obtained in sham-control gastric mucosa. Cross indicates a significant change as compared with the value obtained in rats without treatment with SPT.

CGRP-(8–37), on gastric lesions induced by standard ischemia/reperfusion and accompanying changes in the gastric blood flow are shown in Figs. 6 and 7. Capsaicin denervation increased significantly the ischemia/reperfusion-induced gastric lesions and this effect was accompanied by a marked fall in the gastric blood flow, while pretreatment with CGRP-(8–37) (100 μg/kg, i.p.) by itself failed to influence significantly the ischemia/reperfusion-induced gastric lesions and the gastric blood flow. The pretreatment with standard preconditioning before ischemia/reperfusion caused usual significant decrease in area of gastric lesions and the rise in the gastric blood flow, but failed to affect these parameters in rats with sensory nerve deactivation attained with pretreatment with capsaicin and in those pretreated with CGRP receptor antagonist (Figs. 6 and 7). Addition of CGRP to capsaicin denervation restored the protective effect with an accompanying increase in the gastric blood flow evoked by short ischemia against lesions caused by ischemia/reperfusion (Fig. 7).

3.5. Effect of exogenous adenosine and suppression of adenosine receptors by 8-phenyl theophylline on the gastric lesions induced by ischemia / reperfusion with or without short ischemia

Pretreatment with adenosine (10 mg/kg, i.g.) attenuated significantly the lesions induced by regular ischemia/reperfusion and increased the gastric blood flow with the extent similar to that observed with standard preconditioning (Fig. 8). An nonselective antagonist of adenosine receptors, 8-*p*-sulphophenyl theophylline (10 mg/kg, i.g.), which by itself failed to influence the area of gastric lesions and accompanying increase in the gastric blood flow, reduced significantly the protection and rise in the gastric blood flow caused by both gastric preconditioning or pretreatment with exogenous adenosine against lesions induced by ischemia/reperfusion (Fig. 8).

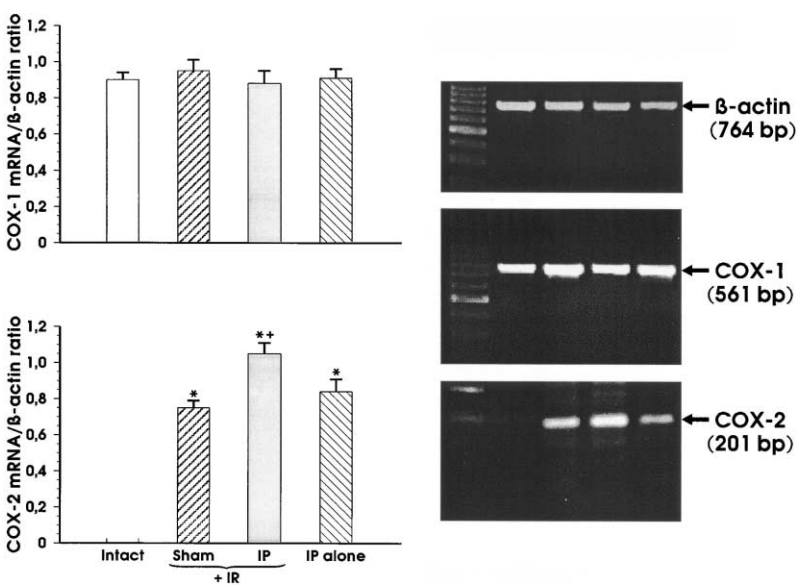

Fig. 9. Messenger RNA expression for β-actin, cyclooxygenase (COX)-1 and cyclooxygenase(COX)-2 mRNA (right panel) and assessment of mucosal gene expression for COX-1 and COX-2 by the intensity of COX-1, COX-2 mRNA/β-actin mRNA ratio in intact gastric mucosa (lane 1), sham plus regular ischemia/reperfusion (I/R) (lane 2), standard ischemic preconditioning (IP) plus regular I/R (lane 3) and IP alone (lane 4). M-size marker DNA, Arrow—expected PCR product (bp). Asterisk indicates a significant change as compared with the value obtained in intact gastric mucosa. Cross indicates a significant change as compared with the value obtained in sham- and IP alone treated animals.

3.6. Expression of cyclooxygenase-1 and cyclooxygenase-2 mRNA by RT-PCR and Western Blot in gastric mucosa exposed to ischemia / reperfusion lesions with or without standard preconditioning

Fig. 9 (right panel) shows expression of β-actin, cyclooxygenase-1 and cyclooxygenase-2 mRNA in the gastric mucosa of intact rats treated with vehicle and not exposed to regular ischemia/reperfusion or those exposed to ischemia/reperfusion with or without standard ischemic preconditioning and killed immediately after the end of regular ischemia/reperfusion. The expression of β-actin mRNA was well preserved in the mucosal samples taken both from rats treated with vehicle (control) or exposed to ischemia/reperfusion and tested at various time intervals (Fig. 9). The cyclooxygenase-1 mRNA was detectable in the vehicle-treated gastric mucosa as well as in the mucosa exposed to regular ischemia/reperfusion at all time intervals after the end of ischemia/reperfusion. Ratio mRNA COX-1 over β-actin revealed that the expression of cyclooxygenase-1 mRNA was similar at all time interval after termination of ischemia/reperfusion (Fig. 9, left panel). In contrast, the signal for cyclooxygenase-2 mRNA was not detected in vehicle control animals, but has been traced in rats exposed to ischemia/reperfusion and in those treated with short ischaemia prior to exposure to ischemia/reperfusion (Fig. 9, right panel). The ratio of cyclooxygenase-2 mRNA over β-actin mRNA showed the expression of cyclooxygenase-2 in preconditioned mucosa was significantly higher than that recorded in animals immediately after the end of ischemia/reperfusion without gastric preconditioning (Fig. 9, left panel).

In the intact rats, cyclooxygenase-2 mRNA and cyclooxygenase-2 protein were not detected, while in the mucosa exposed to regular ischemia/reperfusion with or without standard ischemic preconditioning, the detectable signals for cyclooxygenase-2 mRNA and the expected ~ 72 kDa of cyclooxygenase-2 protein were observed (Fig 10, right panel). The ratio of cyclooxygenase-2 protein to β-actin reached a significantly higher value in preconditioned gastric mucosa than that recorded in sham-operated control (Fig. 10, left panel). The highest gene expression was noticed with the combination of ischemic preconditioning plus regular ischemia/reperfusion.

4. Discussion

This study demonstrates that preconditioning of the gastric mucosa with short episodes of ischemia exerts significant protection against lesions caused by longer exposure to regular ischemia/reperfusion and this protective effect depends upon the time of ischemic episodes. To our best knowledge, this is the first demonstration that the phenomenon of preconditioning described originally in other organs, such as heart, kidney, liver and intestine (Murry et al., 1986; Mounsey et al., 1992; Davenpeck et al., 1994; Parratt, 1994; Russell et al., 1996; Ishida et al., 1997; Nilsson et al., 2000; Peralta et al., 2000), occurs also in the stomach, resulting in the limitation of severe mucosal damage evoked by the ischemia/reperfusion. Our study indicates that gastric preconditioning may represent one of the most powerful protective interventions against

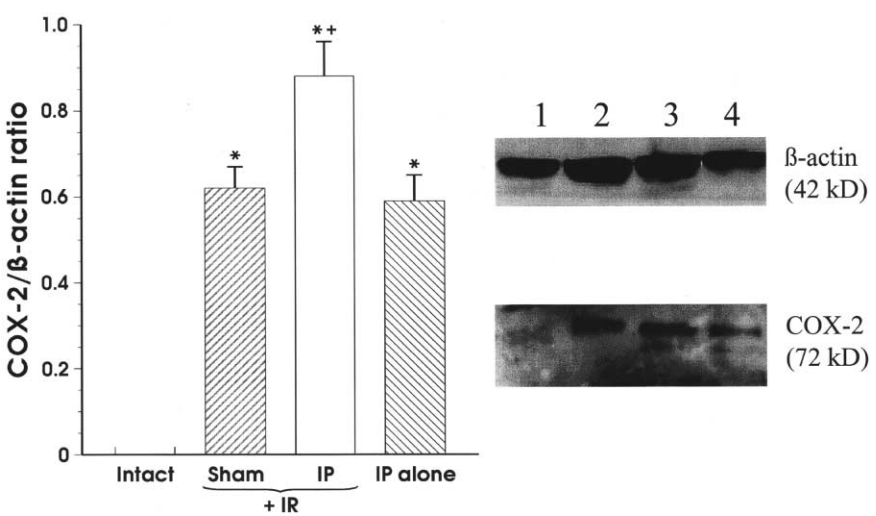

Fig. 10. Representative Western Blot analysis of COX-2 protein and β-actin protein (right panel) and ratio of COX-2 protein over β-actin protein (left panel) in intact rats and those pretreated with standard preconditioning (IP) with or without subsequent exposure to regular ischemia/reperfusion (I/R). Comparisons between the groups were made by determining the COX-2/β-actin ratio by densitometry. Asterisk indicates a significant change as compared with the value obtained in intact gastric mucosa. Cross indicates a significant change as compared with the value obtained in sham- and IP alone treated animals.

the damage induced by severe ischemia/reperfusion, as well as a variety of necrotizing substances, including 100% ethanol in the stomach.

Based on our results, it is reasonable to assume that gastric preconditioning involves several mediators, including prostaglandin derived from cyclooxygenase-1 and cyclooxygenase-2 activity, NO, sensory nerves and adenosine that appear to cooperate in the mechanism of this protection probably by causing vasodilatation and enhancement of the gastric blood flow. This notion is supported by the fact that protection and accompanying rise in the gastric blood flow induced by gastric preconditioning were significantly attenuated by inhibition of cyclooxygenase-1 and cyclooxygenase-2, by L-NNA suppressing NO-synthase activity and by capsaicin ablating functionally sensory nerves that were shown to release NO and various vasodilatatory neuropeptides, such as CGRP. The involvement of the above mediators is supported by the effect of concurrent treatment with synthetic prostaglandin E_2 to compensate for the deficiency of endogenous prostaglandin, by L-arginine to provide a substrate for NO synthase or with exogenous CGRP to replace this sensory neuropeptide lost by deactivation with capsaicin of afferent nerves. Furthermore, we found that the protective and hyperemic effects of preconditioning against ischemia/reperfusion were antagonized by 8-*p*-sulphophenyl theophylline, an antagonist of adenosine receptors, and that exogenous adenosine attenuated significantly gastric lesions induced by ischemia/reperfusion with the extent similar to that observed after standard ischemic preconditioning, suggesting that adenosine may also contribute to the beneficial effect of gastric preconditioning in the stomach.

Previous studies demonstrated that prostaglandin, applied exogenously or generated endogenously in the gastric mucosa, exhibit high activity in preventing the mucosal damage induced by necrotizing substances, including boiling water (Konturek et al., 1982; Robert et al., 1983). Adaptive cytoprotection was introduced originally by Robert et al. (1983) to describe the protective activity of endogenous prostaglandin generated within gastric mucosa by mild topical irritants, such as 20% ethanol or 5 mM NaCl, against severe mucosal damage induced by strong irritants, such as 100% ethanol or 25% NaCl. We demonstrated previously (Konturek et al., 1982) that mild irritants offer the cross-protective response, e.g. 5% NaCl was effective in attenuation of damage induced not only by necrotizing 25% NaCl, but also by 100% ethanol, while 20% ethanol prevented the damage caused by 25% NaCl.

Besides prostaglandin, NO was later on implicated as mediator of this adaptation (Mercer et al., 1992; Cho et al., 1994; Brzozowski et al., 1996a; Ko and Cho, 1999). In fact, some reports suggested that prostaglandin may not be primary mediators of this mucosal adaptive protection (Hawkey et al., 1988). It is of interest that this protective mucosal mild irritation was proposed to act locally because certain mild irritants that are highly protective when applied to the mucosa failed to exhibit any protective activity when applied systemically (Konturek et al., 1982).

Preconditioning refers to a phenomenon in which a tissue is rendered resistant to the deleterious effects of severe and prolonged ischemia followed by reperfusion by previous exposures to brief periods of vascular occlusion (Ishida et al., 1997). While the beneficial effects of preconditioning were first demonstrated in the myocardium (Murry et al., 1986), it is now evident that this preconditioning protects against postischemic damage of brain, kidney, skeletal muscle and gastrointestinal organs, including small bowel and liver (Murry et al., 1986; Mounsey et al., 1992; Parratt, 1994; Tsuruma et al., 1996; Bouchard and Lamontagne, 1996; Peralta et al., 1998). The mechanism of protection induced by preconditioning has not been fully explained, but activation of adenosine A_1 receptors and ATP-sensitive potassium channels in the heart, as well as an inhibition of neutrophil activation and emigration in the intestine, were implicated in this phenomenon (Ishida et al., 1997). No attempts were made, however, to examine whether preconditioning in the stomach could enhance its mucosal resistance against the damage induced by subsequent exposure to prolonged and severe ischemia/reperfusion.

In the present study, we compared the effect of preconditioning on the gastric mucosa injured by ischemia/reperfusion with that exhibited by mild irritants. It was confirmed that gastric mucosa pretreated with mild irritants, such as 5% NaCl, 20% ethanol or 5 mM taurocholate, acquires a tolerance against subsequent damaging insults of strong irritants, such as 25% NaCl, 100% ethanol of 80 mM taurocholate, and we found for the first time that this increased tolerance was also achieved by gastric preconditioning. Furthermore, we have demonstrated for the first time that preconditioning also protected gastric mucosa against lesions induced by strong irritants, such as 100% ethanol, 25% NaCl and 80 mM taurocholate, indicating that preconditioning, like mild irritants (Konturek et al., 1982; Robert et al., 1983), affords cross-protection against lesions caused by these strong irritants.

Numerous studies have documented that prostaglandin derived from the activity of the cyclooxygenase isoforms, especially cyclooxygenase-1, plays an important role in mechanism of gastric integrity, gastroprotection (Konturek et al., 1982; Robert et al., 1983; Vane and Botting, 1995) and ulcer healing (Konturek et al., 1992). Recently, prostaglandin derived from cyclooxygenase-2 were implicated in the protective and ulcer-healing activities of growth factors by the demonstration that cyclooxygenase-2 is upregulated in the edge of gastric ulcer and that this is significantly enhanced by the treatment with growth factors (Brzozowski et al., 2000). Moreover, endogenous prostaglandin derived from cyclooxygenase-1 and cyclooxygenase-2 are involved in the mechanism of mucosal recovery from ischemia/reperfusion-induced acute gastric erosions that

subsequently progressed into deeper ulcerations and that healing of these ulcers is associated with an overexpression of cyclooxygenase-2 mRNA (Brzozowski et al., 1999). The involvement of prostaglandin in the mechanism of preconditioning of other organs has not been fully elucidated, but in case of heart, it was suggested that certain prostaglandin, such as prostacyclin, which is released from ischemic myocardium, limits the extent of heart infarct and attenuates ventricular arrhythmia and that inhibition of cyclooxygenase prevents the protective effect of ischemic preconditioning in dog myocardium (Murry et al., 1986; Parratt, 1994). Our results with the preconditioning in the stomach are in keeping with these findings by showing directly the cyclooxygenase-2 overexpression in the preconditioned gastric mucosa at the levels of both mRNA and protein, while cyclooxygenase-1 mRNA remains unchanged. Moreover, the suppression of the prostaglandin biosynthesis by nonselective (indomethacin) and selective cyclooxygenase-1 (resveratrol) or cyclooxygenase-2 (Vioxx) inhibitors attenuated or abolished the protective and hyperemic effects of gastric preconditioning. Furthermore, the minute amounts of synthetic prostaglandin E_2 analog added to these cyclooxygenase inhibitors restored the protection by preconditioning, reinforcing the notion that endogenous prostaglandins produced in excessive amounts by cyclooxygenase-2, play an important role in the mechanism of gastric preconditioning.

Previous studies revealed that NO released from vascular endothelium, sensory afferent nerves or gastric epithelium is essential for the gastroprotection and ulcer healing (Green et al., 1981; Takeuchi et al., 1994; Whittle, 1994; Whittle et al., 1990; Brzozowski et al., 1996b, 1997a,b). We documented previously that administration of NO-synthase inhibitors abolished the gastroprotective activity of capsaicin in the stomach and delayed healing of chronic gastric ulcers (Brzozowski et al., 1996b). Our present study implies that NO could also participate to the mechanism of gastric preconditioning.

In agreement to previous findings that NO derived from L-arginine metabolic pathway could contribute to the mechanism of preconditioning in the liver injury induced by hepatic ischemia/reperfusion (Nilsson et al., 2000; Peralta et al., 2000), we found that the gastroprotection afforded by gastric preconditioning is accompanied by the rise in the gastric blood flow probably due to enhanced production of NO in the gastric mucosa. Both these effects occurring after preconditioning were almost completely abolished in rats with suppressed NO-synthase activity by L-NNA. Similar reversal of protective effects of gastric preconditioning against ischemia/reperfusion was observed in rats with capsaicin-induced deactivation of sensory nerves, suggesting that NO derives from sensory nerves to afford the protection of short ischemic episodes against severe lesions caused by prolonged ischemia/reperfusion. The important role of NO is further supported by the finding that addition of L-NNA to L-arginine, the substrate for NOS activity, but not D-arginine, restored the gastroprotection against ischemia/reperfusion, luminal release of NO and the hyperemia evoked by gastric preconditioning. Moreover, coadministration of CGRP, together with gastric preconditioning in rats with functionally ablated sensory nerves, restored the gastroprotective and hyperemic effects of this preconditioning with the extent comparable to that achieved with preconditioning in rats with intact sensory innervation. This suggests that the release of neuropeptides, such as CGRP, from sensory afferents may mediate the gastroprotection against the ischemia/reperfusion damage induced by preconditioning. This is in keeping with original observation of Ferdinandy et al. (1997), suggesting that capsaicin-sensitive afferent nerves are also involved in the cardiac preconditioning.

We also tested the hypothesis, suggested by others in hepatic preconditioning (Peralta et al., 1998), that adenosine plays a crucial role in the mechanism of gastric preconditioning. Indeed, the beneficial effects of preconditioning were blocked, at least in part, by the administration of nonselective adenosine receptor antagonist 8-*p*-sulphophenyl theophylline. Furthermore, the pretreatment with adenosine in nonpreconditioned animals resembled the protective effect of preconditioning against the ischemia/reperfusion gastric injury. These observations are consistent with the hypothesis that locally released adenosine during preconditioning might trigger the gastroprotection afforded by preconditioning via activation of adenosine receptors.

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