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ROLE OF PROSTAGLANDINS IN GASTROPROTECTION AND GASTRIC ADAPTATION

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Since Robert discovery that pretreatment with prostaglandin (PG) applied in non-antisecretory dose can prevent the injury of gastric mucosa induced by necrotizing agents, much attention was paid to the role of these cyclooxygenaxe (COX) products in the mechanism of gastric mucosal integrity and ulcer healing. The ability of exogenous PG to attenuate or even completely prevent mucosal damage caused by corrosive substances such as absolute ethanol, hiperosmolar solutions or concentrated bile has been termed "cytoprotection". Increased generation of endogenous PG in the gastric mucosa exposed to the topical contact with "mild irritant" such as 20% ethanol, 1 mM NaCl or 5 mM taurocholate also prevented gastric injury caused by strong irritants *via* phenomenon of adaptive cytoprotection. Other mediators such as growth factors, nitric oxide (NO) or calcitonin gene related peptide (CGRP) as well as some gut hormones including gastrin and cholecystokinin (CCK), leptin, ghrelin and gastrin-releasing peptide (GRP) have been also found to protect gastric mucosa against the damage induced by corrosive substances. This protective action of gut hormones has been attributed to the release of PG or activation of sensory nerves because it could be abolished by the pretreatment with indomethacin or large neurotoxic dose of capsaicin and restored by the addition of exogenous PGE_2 or CGRP, respectively. Short (5 min) ischemia of the stomach applied before prolonged ischemia-reperfusion (I/R) attenuated markedly the gastric lesions produced by this I/R and also prevented the mucosal damage provoked by necrotizing substances. This protection could be abolished by the pretreatment with non-steroidal anti-inflammatory drugs (NSAID) and was accompanied by an enhamcement of gastric mucosal COX-2 expression and activity. Exposure of gastric mucosa to single insult of acidified aspirin (ASA) causes severe mucosal damage with occurence of multiple haemorrhagic lesions but with repeated application of ASA, the attenuation of mucosal lesions is observed, despite the profound inhibition of PGE_2 generation. This phenomenon called "gastric adaptation" does not appear to depend upon endogenous biosynthesis of PG but possibly involves enhanced production of growth factors increasing cell proliferation and mucosal regeneration. Unlike short lived gastroprotection by PG, NO, CGRP, mild irritants or short ischemia, gastric

adaptation appears to be long-lasting phenomenon accompanied by increased resistance of the adapted mucosa to subsequent damage induced by corrosive agents.

INTRODUCTION

Role of exogenous and endogenous PG in the mechanism of gastric mucosal integrity and gastroprotection

It is well known that the stomach can defend himself from the injury caused by a variety of strong topical irritants and obnoxious agents due to the activation of several lines of defense, among them the most important being protective mucus and bicarbonate secretion, mucosal hydrophobicity, gastric microcirculation, generation of protective prostaglandins within gastric mucosa, increase in the mucosa sulfhydryls and release of vasoactive neuropeptides from sensory nerve afferents. In 1979, the phenomenon of "cytoprotection" was introduced into the literature by Andre Robert (1), who described the unexpected and fascinating finding that prostaglandins (PG), the major products of arachidonate metabolism through cyclooxygenase activity can be crucial for the maintenance of the gastric integrity. He provided the experimental evidence that PG when applied exogenously in the non-antisecretory doses, exhibit high activity in preventing the mucosal damage induced by necrotizing substances such as ethanol, hiperosmolar solutions, strong acids (e.g. 0.6 N HCl), base (e.g. 0.2 N NaOH) and concentrated bile including even the lesions caused by boiling water (1) (*Fig.1*). The precise mechanism of cytoprotective action of prostaglandins remained unknown but this stimulatory action of these agents on gastric mucus and bicarbonate secretions, an increase in the gastric microcirculation and the enhancement in the mucosal sulfhydryl compounds were initially proposed to explain this phenomenon. We were able confirmed not only this finding but also documented, for the first time, that certain growth factors, especially EGF, could be considered as gastroprotective because they were also capable of reducing aspirin-induced gastric ulcerations in rats and cats under the conditions where biosynthesis of endogenous PG was completely inhibited by the administration of this NSAID (2).

Phenomenon of adaptive cytoprotection mediated by prostaglandins

It became evident that the one of the important forms of cytoprotection is "adaptive cytoprotection", the term that was also introduced originally by Robert and his associates (3) to describe the protective activity of endogenous

Cytoprotection by PGE$_2$ against various necrotizing substances
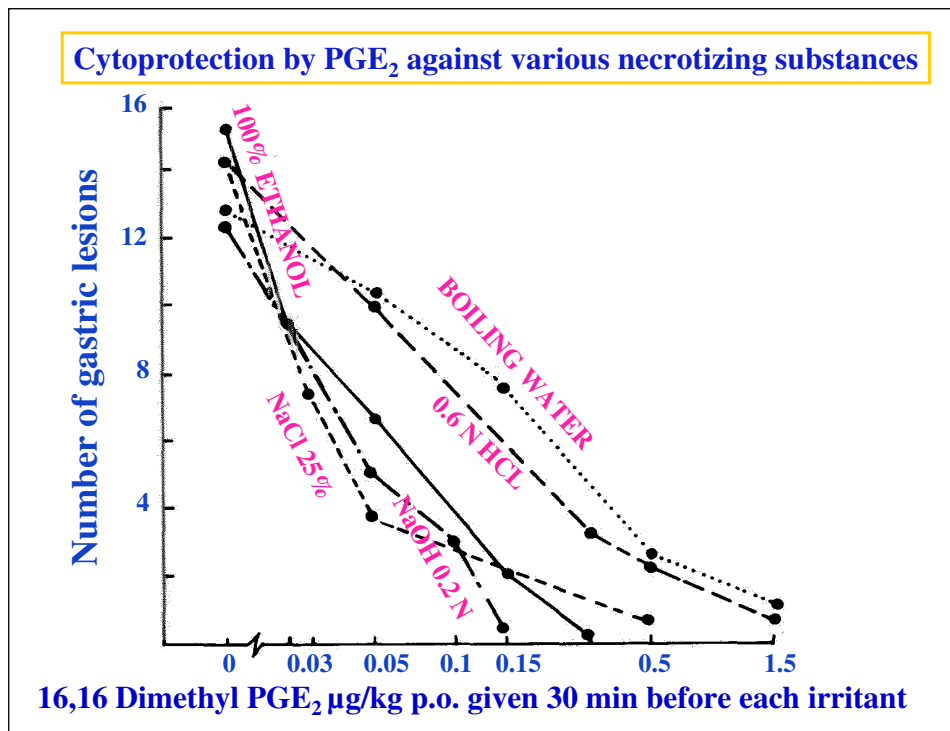
Fig.1. The effect of i.g. pretreatment of PGE$_2$ methyl analog applied i.g. in graded doses ranging from 0.03 up to 1.5 µg per rat, on the on the number of gastric lesions induced by necrotizing agents such as 100% ethanol, 25% NaCl, 0.2 n NaOH, 0.6 N HCl or boiling water (1 ml/rat). Mean ± SEM of 4 per each experimental group. Minute amount of exogenously applied methyl PGE$_2$ analog was capable of attenuating of these lesions almost completely.

prostaglandins generated within gastric mucosa by mild topical irritants such as 20 % ethanol, 5 mM NaCl or 5 mM taurocholate in response to severe mucosal damage induced by strong irritants such as 100 % ethanol, 25 % NaCl or 80 mM taurocholate (*Fig. 2*). The concept of cytoprotection pioneered by Robert's experimentation's was further extended by the observation 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 100% ethanol or 25% NaCl (4). Moreover, using the bioassay technique to measure a generation of prostacyclin (PGI$_2$) and PGE$_2$ in the gastric mucosa, our group found that the pretreatment of gastric mucosa with mild irritant resulted in an enhancement of the mucosal generation of PGI$_2$ and PGE$_2$, thus providing direct evidence for the involvement of endogenous PG in the mechanism of adaptive cytoprotection (4) (Fig. 3). We proposed that this protective mucosal mild-irritation could be attributed to the local action of endogenous PG because mild irritants failed to
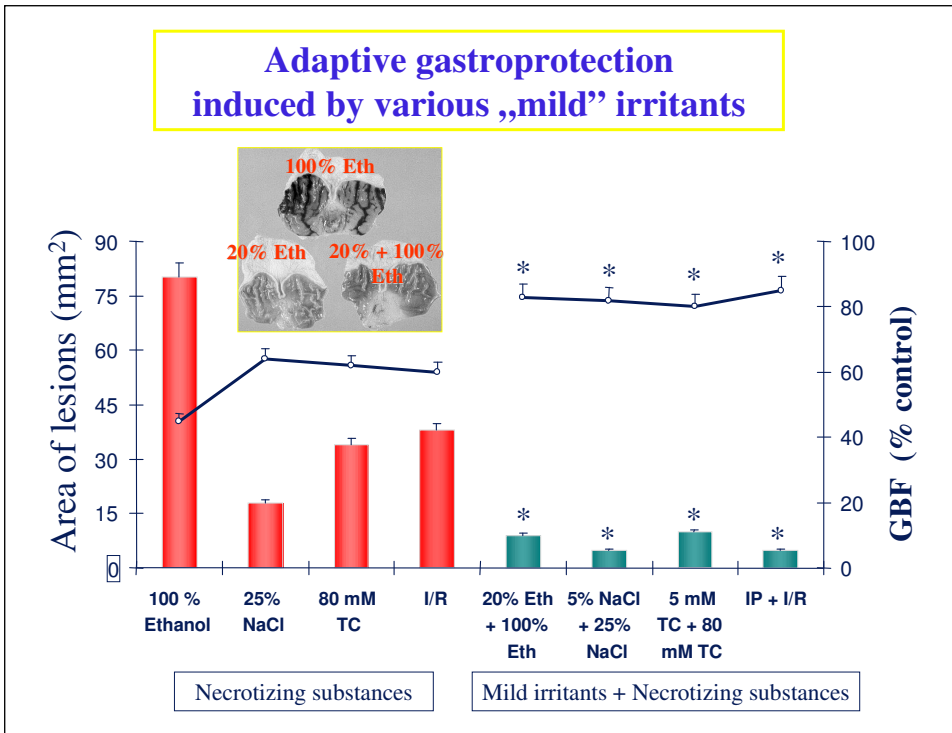
Fig. 2. Comparison of the effect of various mild irritants (20% ethanol, 5% NaCl and 5 mM taurocholate) applied i.g. and short ischemic preconditioning (IP) induced by 2 times 5 min occlusion of the rat celiac artery, on the area of gastric lesions and alterations in the GBF induced by i.g. topical application of necrotizing substances (100% ethanol, 25% NaCl or 80 mM TC) or the exposure of gastric mucosa to 30 min of ischemia followed by 3 h or reperfusion (I/R). Short ischemic preconditioning mimics the gastroprotective effect of mild irritants against lesions provoked by necrotizing agents resulting in a significant attenuation of the gastric mucosa injury induced by prolonged I/R. Mean ± SEM of 6-8 rats. Asterisk indicates a significant change as compared to the value obtained in rats without pretreatment with mild irritants or IP.

exhibit any protective activity when applied systemically (4,5). It is of interest that exogenous PGE_2 exhibited cytoprotective activity against the damage induced by ethanol and indomethacin to the isolated gastric mucosal cells and gastric glands *in vitro* (6-8) indicating that this cytoprotective activity of PG *in vitro* conditions may contribute, at least in part, to the gastric protection observed in the stomach pretreated with PG *in vivo*. These studies supported the notion that PG possessed the ability to directly attenuate the cell damage without the contribution of neural and hormonal factors as well as gastric mucosal circulation (8). This PG mediated cytoprotection in isolated cell systems has been a controversial subject because some experimental evidence suggested that PG protection does not exist *in vitro* and questioned also the notion that PG are

primary mediators of adaptive cytoprotection. Instead of primary mediatory role of PG in adaptive cytoprotection, other mechanisms were emphasized including enhanced gastric blood flow and stimulation of mucus release in the gastric mucosa due to the local irritating effect to of the mild irritant (9-11). Moreover, it was suggested that the partial reversal of adaptive cytoprotection by indomethacin, an inhibitor of PG biosynthesis, could be secondary to the some other action of this agent such as reduction in gastric blood flow rather than the direct effects on prostanoid synthesis enhanced in response to mild irritant (12).

Non-prostaglandin mechanism of gastric mucosal protection and mucosal restitution following injury

The promise that potential pharmacological formulations containing PG may exert therapeutic efficacy against mucosal injury and in peptic ulcer disease in clinical settings had however, not been fulfilled. First, the potential clinical application of cytoprotective PG included not only their prevention of acute
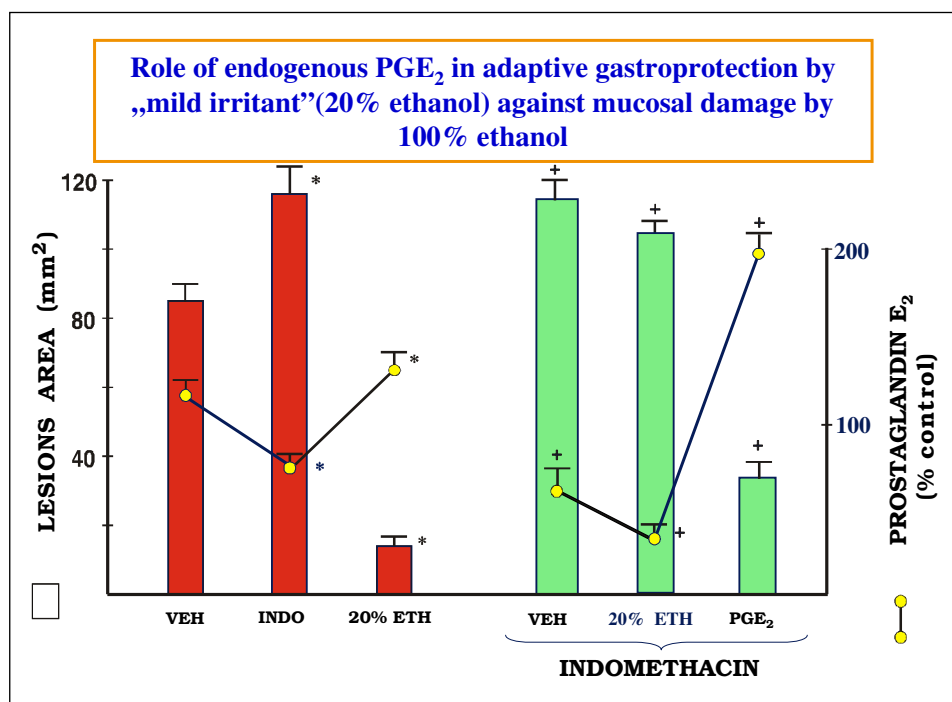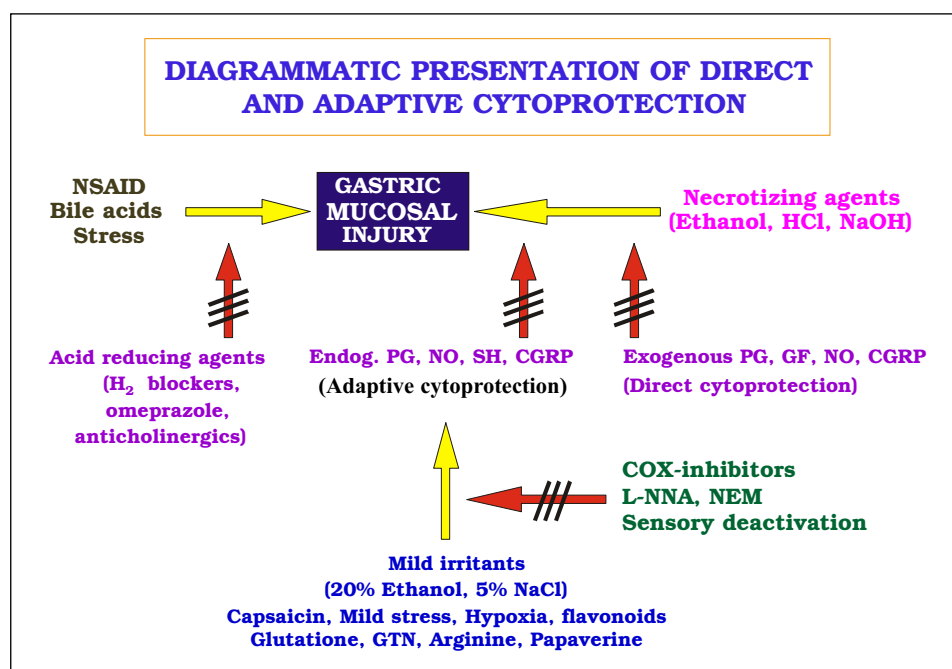
Fig. 3. Effect of intragastric (i.g.) application of vehicle, mild irritant (20% ethanol; 1 ml/rat) or PGE_2 (5 µg/kg) without or with concurrent administration of indomethacin (5 mg/kg i.p.) on the area of gastric lesions induced by the topical application of 100% ethanol and accompanying changes in the generation of PGE_2 in the gastric mucosa. Mean ± SEM of 6-8 rats. Asterisk indicates a significant change as compared to the value obtained in animals treated with vehicle. Cross indicates a significant change as compared to the value obtained in rats without pretreatment with indomethacin.

gastritis such as those caused by alcohol, aspirin and other NSAID or by biliary reflux but also the mechanism of inhibition of gastroduodenal disordes such as reflux esophagitis, peptic ulcer disease, ulcer recurrence and gastritis associated with gastric ulcer. It become quickly evident that PG at non-antisecretory doses can not accelerate ulcer healing being also ineffective in the prevention of ulcer recurrence and reflux esophagitis. Second, by definition, PG were originally implicated in cytoprotection of the all layer of the gastric mucosa against the damage induced by noxious-necrotizing substances but then it became apparent from detailed histological assessments of the gastric mucosa "protected" from the acute gastric injury by PG that these arachidonate metabolites failed to prevent morphologic disruption of surface epithelium and cell desquamation after ethanol administration (13). Although PG prevented the macroscopic injury induced by ethanol, they were not capable to prevent the destruction by this agent of superficial epithelial gastric mucosal cells but enhanced rapid restitution of the damaged mucosa by stimulation of mucosal cell migration from the intact foveolar and neck-gland area (13,14). The fact that the PG afforded protection to the deeper mucosal layers predominantly including regenerative zone of gastric glands, but failed to prevent injury to the superficial mucosal cells, turned however, into the question their "truly" cytoprotective properties (14,15).

The process of rapid repair or restitution of the gastric mucosa occurs to reestablish epithelial continuity and barrier function after injury. Restitution was first described *in vitro* in the bullfrog gastric mucosa (16) but, at present, it is considered as a more generalized response to the superficial injury along the GI tract (17,18). By definition restitution means the rapid re-epithalization after superficial gastric injury that is caused by migration of persisting viable epithelial cells from the areas surrounding the damage (16). In 1984, Ito et al. (17) showed for the first time, that the ethanol damage to rat gastric mucosa led to 99% necrotic destruction of luminal surface of the gastric mucosa within 30-45 sec but this damaged area started to restitute rapidly due to extensive cell migration. Furthermore, restitution that requires also the energy from aerobic glycolysis, to drive the migration of cells at the apical surface of the mucosa, was shown to be completed within 4 h in amphibian gastric mucosa (18). Studies by Ito at al. (17) provided morphologic and physiological evidence that rapid restitution consists of two-part processes. First, uninjured cells became flattened, extend lamelopodia and migrate from confluent sheet of epithelial cells at the apical surface of the mucosa. Second, the monolayer of flattened cells then reestablish tight junctions and cell polarity to restore barrier functions. Since PG treatment failed to prevent initial morphologic damage, even exerting a stimulatory effect on rapid restitution process, it was concluded that the protective action of these arachidonate products could not be attributed to their genuine "cytoprotective" activity. Furthermore, it was proposed that adequate Ca^{+2} and bicarbonate concentrations play a major role in the mucosal restitution after the damage induced by hyperosmolar solution because removal of Ca^{+2} from the medium or substitution of neutral buffer

(HEPES) for HCO_3^- in the their gastric mucosa mounting system, markedly impaired the restitution of the bullfrog gastric mucosa mounted in Ussing chamber *in vitro* (18). Studying other possible mediators of restitution, Paimela *et al.* (19) have indicated that growth factors such as bFGF can mediate microscopic and electrophysiological recovery from the mucosal damage induced by hyperosmolar solution (1 mM NaCl). The exact mechanism of restitution process remains unknown but recent observation by Hagen *et al.* (20) identified novel pathway Na^+driven HCO_3^- transport that could be involved in restitution, which seems to be independent from Na^+/H^+ exchange and Na^+-K^+-$2Cl^-$ cotransport originally implicated in the ionic mechanism of process of restitution.

Besides PG, another important mediator, nitric oxide (NO), was later implicated as a mediator of adaptive cytoprotection and in fact, some reports suggested that PG might not be a primary mediator of this mucosal adaptive cytoprotection (15,21). The contribution of NO to adaptive cytoprotection was based on the finding that L-NNA reversed the effect of mild irritant with the extent similar to that observed with administration of indomethacin (*Fig. 4*). Furthermore, concurrent treatment with L-arginine, a substrate for the NO-

DIAGRAMMATIC PRESENTATION OF DIRECT AND ADAPTIVE CYTOPROTECTION

NSAID Bile acids Stress → GASTRIC MUCOSAL INJURY ← Necrotizing agents (Ethanol, HCl, NaOH)

Acid reducing agents (H_2 blockers, omeprazole, anticholinergics)

Endog. PG, NO, SH, CGRP (Adaptive cytoprotection)

Exogenous PG, GF, NO, CGRP (Direct cytoprotection)

COX-inhibitors L-NNA, NEM Sensory deactivation

Mild irritants (20% Ethanol, 5% NaCl) Capsaicin, Mild stress, Hypoxia, flavonoids Glutatione, GTN, Arginine, Papaverine

Fig. 4. Scheme summarizing of the effect of necrotizing agents such as ethanol, HCl and NaOH and ulcerogenic compounds and factors such as NSAID, bile acid and stress resulting in gastric mucosal injury and the mechanism of direct and adaptive cytoprotection mediated by protective factors such as PG, growth factors, NO, CGRP and mild irritants (e.g. 20 % ethanol, 5% NaCl) to counteract the damage induced by these ulcerogens.
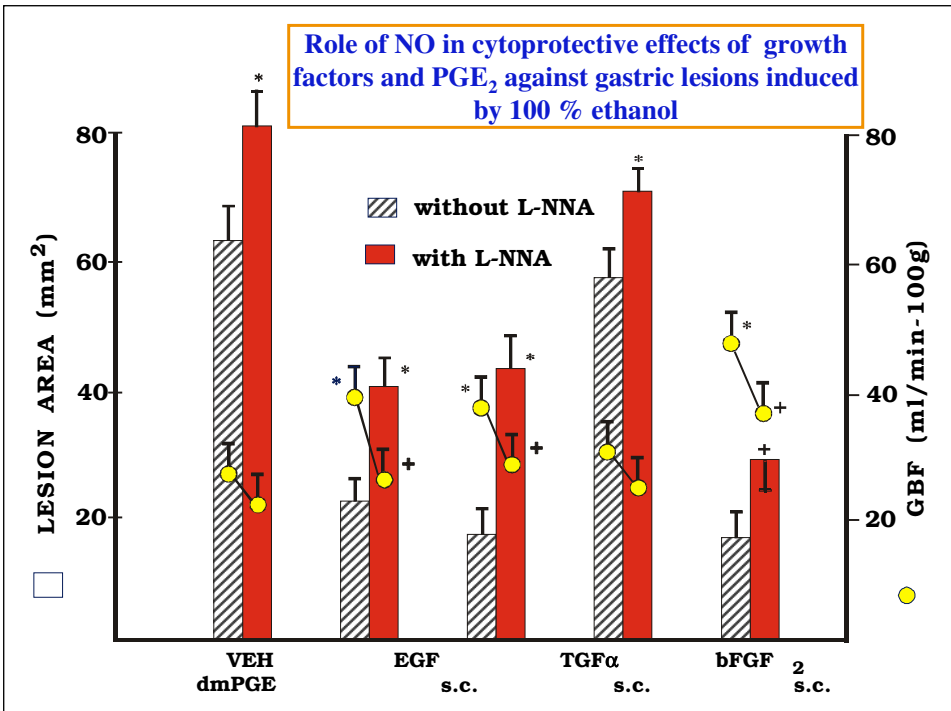
Fig. 5. Effect of vehicle, various growth factors (EGF, TGFα, bFGF applied in a dose of 50 µg/kg s.c.) and 16,16 dimethyl PGE_2 (dm PGE_2; 5 µg/kg i.g.) with or without the pretreatment with L-NNA (20 mg/kg i.p.) on area of gastric lesions induced by 100% ethanol. Suppression of NO-synthase activity by L-NNA significantly attenuated the reduction of gastric lesions caused by growth factors and dmPGE_2. Mean ± SEM of 6-8 rats. Asterisk indicates a significant change as compared to the value obtained in animals treated with vehicle. Cross indicates a significant change as compared to the value obtained in animals without L-NNA pretreatment.

synthase activity, co-administered with L-NNA or when exogenous PGE_2 analog added to indomethacin, they counteracted the inhibitory effect of L-NNA and indomethacin on adaptive cytoprotection induced by 20% ethanol and diminished an increase in the GBF induced by this mild irritant.

Extensive experimental studies in the last decade revealed that NO released from vascular endothelium, sensory afferent nerves, or that originating from gastric epithelium is essential not only for adaptive cytoprotection but also for the gastroprotection evoked by many physiological factors including growth factors such as EGF, bFGF TGFα and PDGF, or gastrointestinal hormones, such as cholecystokinin (CCK), gastrin, leptin and ghrelin (22-27).

EGF when applied subcutaneously, markedly attenuated the gastric lesions evoked by ethanol and the protective activity of this peptide was inhibited by L-NNA, indomethacin, DFMO, an inhibitor of ornithine decarboxylase (ODC)-polyamine pathways (*Fig. 5*). This study have indicated that growth factors may

exert protective effect on the gastric mucosa injured by ethanol *via* mechanism involving mucosal NO and PG as well as enhanced mucosal polyamines and/or sulfhydryls biosynthesis (*Fig. 6*). Furthermore, we documented that gastrointestinal hormones such as CCK and gastrin exhibit a potent gastroprotective activity against necrotizing injury induced by ethanol and mucosal damage caused by aspirin, *via* prostaglandin-independent mechanism (24,25).

The role of satiety hormons, especially ghrelin which recently triggered attention of numerous investigators, in the mechanism of gastric mucosal defense and gastroprotection has been little elucidated except for the report of Sibilia *et al.* (28) who showed recently that central administration of ghrelin reduced the lesions induced by ethanol. This gastroprotective effect of ghrelin was attenuated by the blockade of NOS activity with L-NAME and by the functional ablation of sensory afferent nerves with capsaicin. The question remains whether ghrelin contributes to gastroprotection against gastric lesions caused not only by the artificial irritant such as ethanol but also by natural ulcerogenic conditions such as stress and what is the role for the cyclooxygenase (COX)-PG in the possible gastroprotective effect of this peptide. We found (29) that exposure to water immersion and restraint stress upregulates mRNA for ghrelin in the gastric mucosa suggesting that this hormone may act locally and activate various protective mechanisms and contribute to the maintenance of gastric mucosal defense against damage induced by noxious agents. It is of interest that the
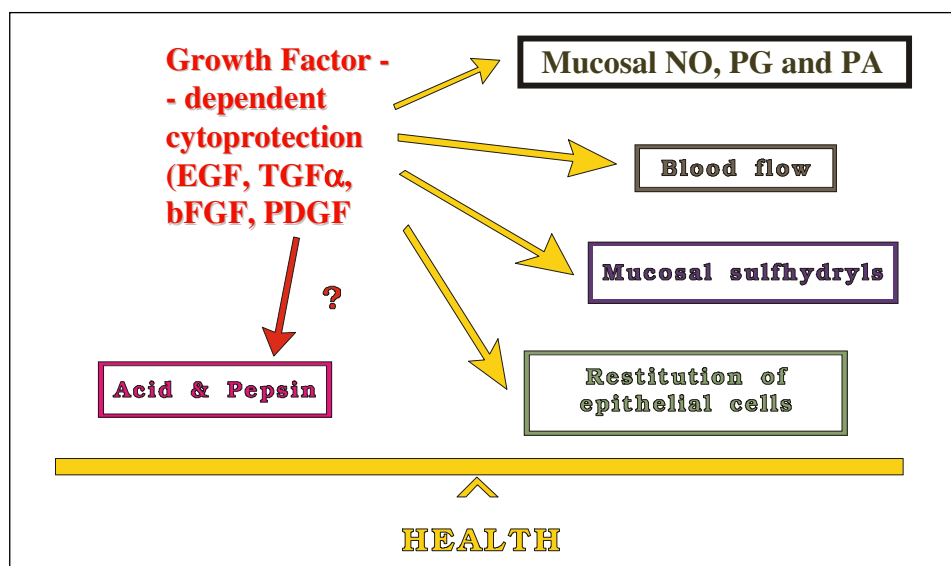
Fig. 6. Possible mediators implicated in the growth factor-dependent cytoprotection. Growth factors such as EGF, TGFα, bFGF and PDGF exhibit gastroprotective action due to activation of mucosal NO, PG and polyamines (PA), increase in the gastric blood flow (GBF) and mucosal sulfhydryls and speeding up process of restitution of gastric epithelial cells.
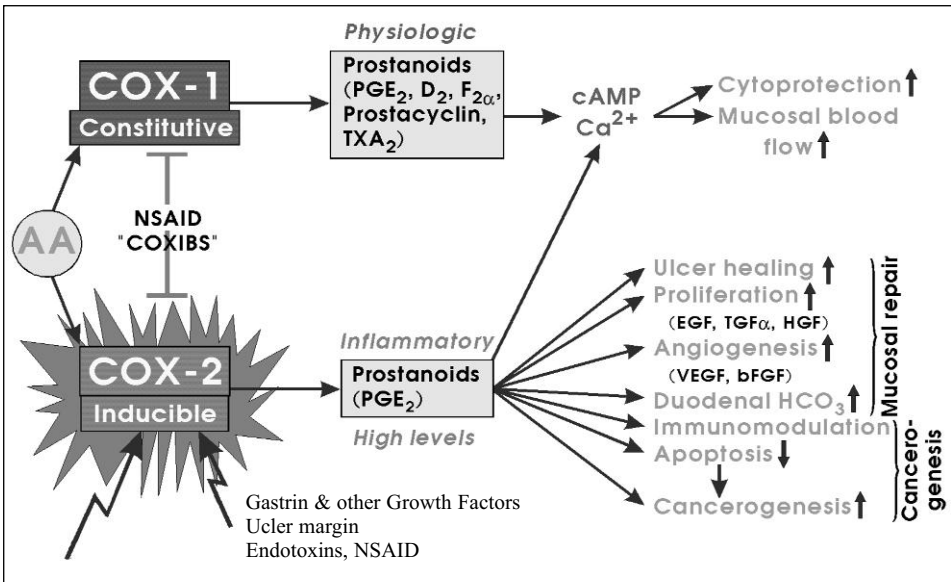
Fig. 7. Schematic characteristics of prostaglandin (PG)-cyclooxygenases (COX)-1 and COX-2 that convert arachidonic acid to unstable endoperoxidase PGG_2 and then to PG. COX-1 is expressed constitutively and releases PGE_2 and PGI_2 (prostacyclin) involved in cytoprotection and accompanying increase in the gastric blood flow (GBF). Another product of COX-1, thromboxane (TXA_2) exhibits vasoconstrictor and anti-platelet activity. COX-2 produces PG and enhances activity of proteases and growth factors increasing cell proliferation and contributing to ulcer healing and mucosal repair *via* enhancement in the bicarbonate secretion and angiogenesis mediated by proangiogenic growth factors such as VEGF and bFGF.

protective and hyperemic effects of central and peripheral ghrelin were completely abolished by vagotomy and significantly attenuated by suppression of COX-1 and COX-2 with indomethacin and rofecoxib supporting the notion that vagal nerves and COX-PG system play an imporatant role in ghrelin-induced protection and accompanying hyperemia.

Implication of cyclooxygenase (COX)-1 and COX-2 products in the mechanism of gastroprotection and gastric adaptation

Recent advances on the enzymatic pathways of arachidonate metabolism revealed that PG synthesis depends upon the activity of cyclooxygenase (COX), a rate-limiting enzyme in the synthesis of eicosanoids (*Fig. 7*). Two isoforms of COX were identified in many cells; a constitutive enzyme designated as COX-1 and inducible isoform known as COX-2 (30). COX-1 appears to be responsible for the production of PG that is physiologically important for homeostatic functions, such as maintenance of the mucosal integrity and mucosal blood flow (31). Under physiological conditions prostanoid synthesis depends upon the

availability of arachidonic acid and the COX-1 activity, that is a major target for nonsteroidal anti-inflammatory drugs (NSAID) causing mucosal damage in the stomach (32). PG derived from the activity of the COX isoforms, especially COX-1, play an important role in mechanism of gastric integrity, gastroprotection and ulcer healing (31,32). Recently, prostaglandins derived from COX-2 were implicated in the protective and ulcer healing activities of growth factors by the demonstration that COX-2 is upregulated on the edge of the gastric ulcer and this is significantly enhanced by the treatment with growth factors (33). Moreover, endogenous prostaglandins derived from COX-1 and COX-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 COX-2 mRNA (32). Our notion that the expression of COX-2 plays an important role in the healing of gastric ulcers remains also in keeping with the observation by Gretzer et al. (34) who reported that PG derived from COX-2, not only from COX-1, may be involved in adaptive cytoprotection induced by a topically applied mild irritant, when a larger area of mucosa is injured.

NSAID such as aspirin (ASA) are widely used because of their well recognized anti-inflammatory, anti-pyrogenic and anti-thrombotic properties, however the major limitation of their clinical application are serious side-effects, including damage of gastrointestinal mucosa, aggravation of stress lesions and exacerbation of pre-existing gastric ulcerations (35). This deleterious action of conventional NSAID was attributed to their topical irritating effect, suppression of gastric mucosal PGE_2 activity, activation of neutrophils, fall in the microcirculation and enhancement in the motility induced by these agents (35).

An interesting, practical, and important discovery related to the gastric damage induced by NSAID is an increase in mucosal tolerance or adaptation to the ulcerogenic action of these drugs that develops with their repeated and more prolonged administration (36-38). This remarkable attenuation of mucosal damage had been first demonstrated in rats (36) and then confirmed in humans (37,38). Initially, aspirin caused a widespread gastric mucosal injury which with repeated ASA application, was followed by the adaptation of the mucosa and increased tolerance to withstand further insult without significant injury (39). Interestingly, this remarkable ability of the gastric mucosa to withstand the prolonged exposure to the ulcerogenic action of aspirin does not depend upon the PG biosynthesis because this generation is suppressed with the first dose of aspirin and remained suppressed during repeated administration of this NSAID (39) (*Fig. 8*). We observed that following ASA ingestion, EGF, which is normally present in saliva and gastric juice, and exerts potent mitogenic and gastroprotective activities, contributed significantly to the increased cellular proliferation in gastric mucosa observed during repetitive ASA insults thus probably playing a major role in the mechanism underlying gastric mucosal adaptation (39). Moreover, the adaptation to repetitive ASA insults was
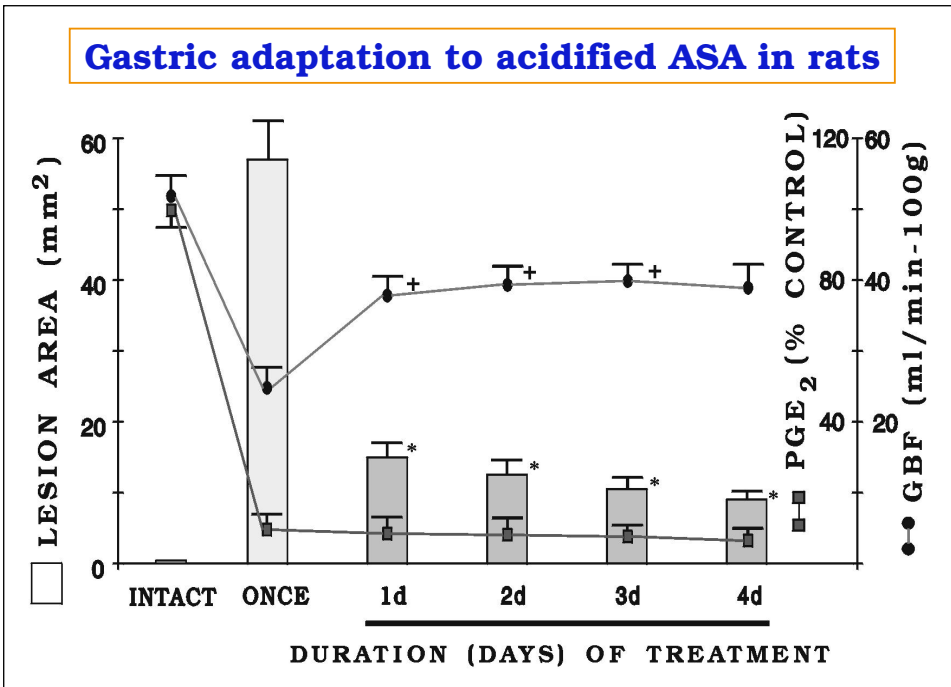
Fig. 8. Development of the gastric adaptation to aspirin (ASA) in rats. Acidified ASA (100 mg/kg) was administered i.g. for the first time (once) and this treatment was repeated subsequently for 4 days. The area of gastric lesions was significantly decreased whereas the GBF was significantly increased in rats treated repeatedly with aspirin despite almost complete suppression of the gastric mucosal generation of PGE_2 in animals exposed to single or repeated administration of acidified ASA. Mean ± SEM of 6-8 rats. Asterisk indicates a significant decrease as compared to the value obtained in animals treated with ASA applied once. Cross indicates a significant change as compared to the value obtained in intact gastric mucosa.

accompanied by the reduction in both the number of circulating neutrophils and the severity of neutrophil infiltration into the gastric mucosa. It is of interest, that the reduction in mucosal neutrophil infiltration and the fall in blood neutrophilia were already seen after the first rechallange with aspirin and it was accompanied by the significant increase of the gastric blood flow in animals and human subjects (39,40). This increase in the gastric blood flow accompanying ASA-induced gastric adaptation was blunted by L-NNA, the inhibitor of NO-synthase, but this inhibitor failed to eliminate gastric adaptation indicating that suppression of NO is essential in the mechanism of the hyperemia but probably is not the major and the only factor in the development of gastric adaptation to repeated treatment with NSAID. This adaptation does not appear to be mediated by endogenous PG, since prolonged administration of ASA was accompanied by almost complete suppression of COX-1 and COX-2 activity in the gastric mucosa

of experimental animals and humans (41,42). Furthermore, our group demonstrated that the rat gastric mucosa adapts not only to topical ulcerogens such as acidified ASA but also to other topical and non-topical abnoxious factors such as ammonia (43) or stress caused under experimental conditions by repetitive exposures to cold and restraint technique (44). It is of interest that the acidified ASA- and stress-adapted gastric mucosa displayed enhanced resistance to subsequent challenges with other topical irritants such as concentrated ethanol, 25% NaCl and diluted bile solutions (*Fig. 9*) *via* mechanism involving enhanced expression and release of EGF and increase in the gastric mucosal cell proliferation triggered in the stomach by repeated ASA insults (41). Furthermore, the reduction in microbleeding rate in ASA adapted patients taking ASA for 14 days was dramatically counteracted in human subjects infected with *Helicobacter*

Gastric adaptation to repeated ASA insults increases the resistance of gastric mucosa to other potent irritants
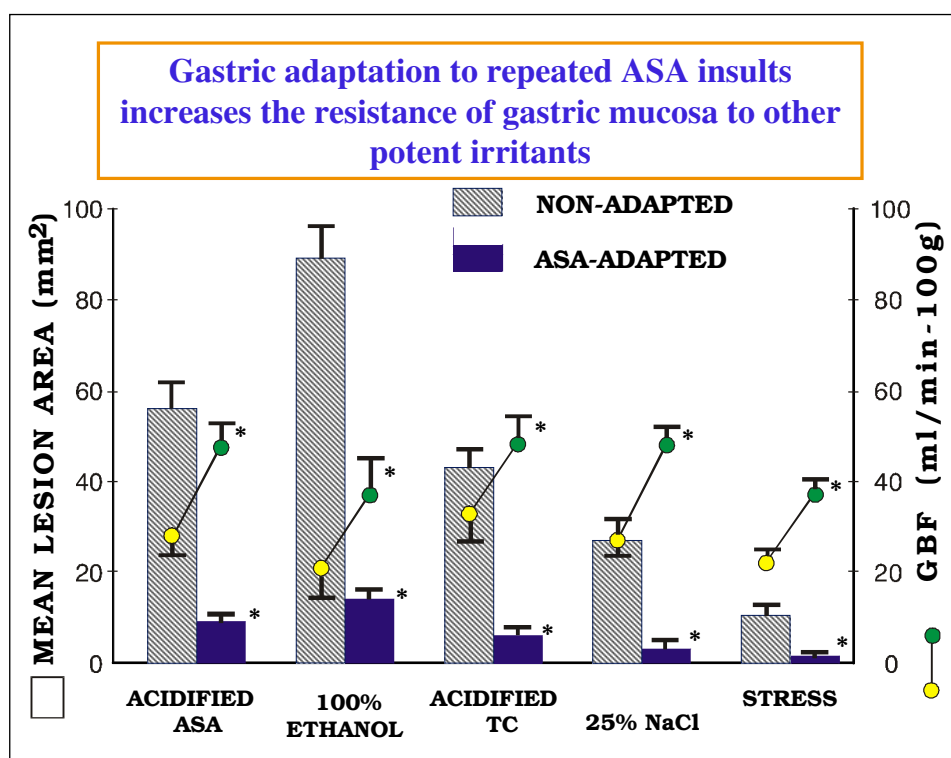
Fig. 9. Effect of single and 4 times daily administration of aspirin (150 mg/kg i.g.) on gastric the mean area of gastric lesions and the accompanying changes in the GBF in rats exposed at 3 h after the last dose of ASA to intragastric (i.g.) treatment with 100% ethanol (1 ml/rat), acidified taurocholate (TC; 80 mM/L), 25% NaCl (1 ml/rat) or to 3.5 h of water immersion and restraint stress (WRS). Gastric mucosa adapted to repetitive ASA treatment shows the enhanced resistance to the damage induced to other potent irritants. Mean ± SEM of 6-8 rats. Asterisk indicates a significant change as compared to the value obtained in non-adapted rats.
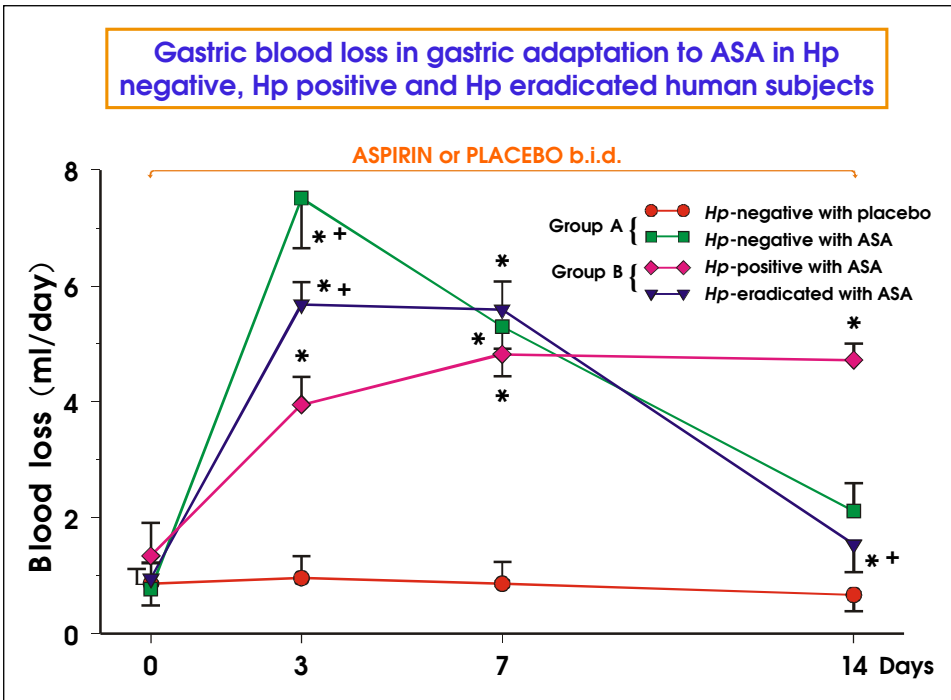
Fig. 10. Influence of *Helicobacter pylori* (Hp) infection on the process of gastric adaptation to 14 day treatment with aspirin (ASA) or placebo, as reflected by the determination of the rate of blood loss (microbleeding) in Hp-negative and Hp-positive patients. The blood loss was maximal at day 3 after the start of ASA ingestion in both, Hp-negative and Hp-positive subjects, but following ASA treatment at day 7 and day 14, it was significantly reduced only in Hp-negative subjects. In contrast, Hp-positive patients demonstrated similar value of blood loss at 3, 7 and 14 days indicating the failure of gastric adaptation to continued ASA administration. Triple eradication anti-Hp therapy in these patients restored the gastric adaptation to continued ASA treatment with the extent similar to that observed in Hp-negative gastric mucosa.

pylori suggesting that this germ can impair the gastric adaptation to continued ASA administration (*Fig. 10*).
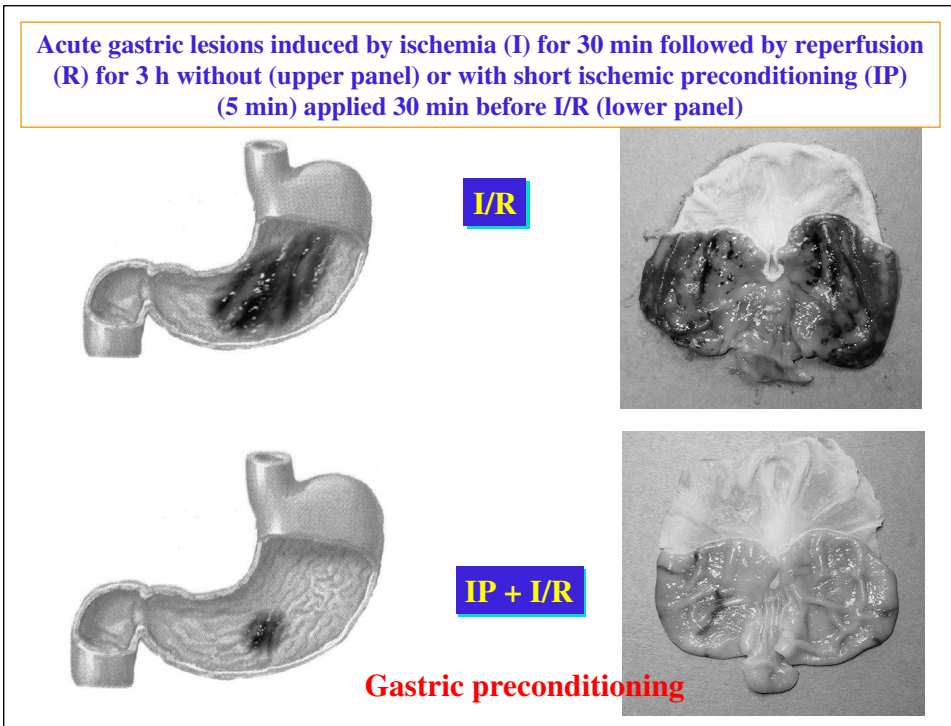
NO releasing NSAID, the new drugs with the ability to spare gastrointestinal tract

Since gastrointestinal ulcerations are associated with the use of all NSAID, a new strategy for the treatment of inflammatory states included a novel series of NSAID that consist of an NSAID linked to a NO-releasing moiety (45). The rationale behind the development of this NO-NSAID composite, was that NO released from this compound would counteract two events that occur subsequent to the suppression of PG synthesis by the NSAID, namely reduced gastric blood flow and an increased adherence of neutrophils to the vascular endothelium of the gastric microcirculation (45-47), thus, sparing the gastric mucosa. For instance

NO-releasing derivative, such as NO-aspirin (NO-ASA) constructed by adding an nitroxy-butyl moiety to aspirin, was found to exhibit lower gastric toxicity despite similar inhibition of both COX-1 and COX-2 activity in the gastric mucosa and exerting anti-thrombotic effects comparable to its parent NSAID (47,48). These NO-releasing NSAID by themselves exhibit only minimal ulcerogenic properties in the gastrointestinal tract, despite exerting a potent anti-inflammatory and analgesic action, similar to native NSAID (45,46). The major importance of NO in the prevention of mucosal damage or in preservation of normal ulcer healing is supported by previous studies showing that both endogenous NO released by capsaicin or NO originating from L-arginine, a substrate for NO-synthase (NOS), or that released from glyceryl trinitrate exert gastroprotective activity, mainly due to hyperemia and the maintenance of blood flow in stressed gastric mucosa (49). We found that classic NSAID such as indomethacin and ASA aggravated acute gastric lesions induced by ethanol and stress mainly due to suppression of endogenous PG, the products of COX-1 and COX-2 activity (48,50). This deleterious action of classic NSAID such as indomethacin or aspirin was accompanied by the impairment in GBF and excessive proinflammatory cytokine, IL-1β and TNF-α expression and release, induced by these NSAID (50,51). The effects of both specific and nonspecific COX-1 and COX-2 inhibitors on stress-induced gastric damage were fully restored by the addition to these inhibitors of PGE$_2$ applied in minute doses which themselves failed to affect the stress-induced gastric lesions (52). All these observations led to the conclusion that the deleterious effect of classic NSAID on stress-induced gastric lesions can be reproduced by selective COX-1 and COX-2 inhibitors suggesting that both COX isoforms are involved in the pathogenesis of stress-induced gastric lesions and the mechanism of mucosal repair and recovery of gastric mucosa from these lesions (46,50-52).

Involvement of prostaglandins in the phenomenon of gastric preconditioning

As mentioned before PG play an important role in the mechanism of gastroprotection and mucosal recovery from the acute gastric lesions but their contribution to the mechanism of short ischemia-induced organ protection, called ischemic preconditioning (53), have been little studied. This ischemic preconditioning refers to a phenomenon in which a tissue is rendered resistant to the deleterious effect of prolonged severe ischemia followed by reperfusion by previous exposures to brief moderate vascular occlusions (54). These protective effects of short ischemia preconditioning were first described in the heart by Murry and coworkers in 1986 (53) but very little evidence was accumulated as to whether similar adaptation to injury induced by ischemia-reperfusion exists in the gut. We have studied this phenomenon in the gastric mucosa subjected to brief 2-5 episodes of short ischemic preconditioning followed by prolonged ischemia-reperfusion that within 3 h causes gross and microscopic erosions in the stomach

Acute gastric lesions induced by ischemia (I) for 30 min followed by reperfusion (R) for 3 h without (upper panel) or with short ischemic preconditioning (IP) (5 min) applied 30 min before I/R (lower panel)

I/R

IP + I/R

Gastric preconditioning

Fig. 11. Macroscopic appearance of gastric lesions in the stomach induced by I/R (upper panel) or ischemic preconditioning (2 times 5 min episodes of short ischemia)(lower panel). Note, that there is prominent reduction in the I/R-induced by gastric lesions in preconditioned gastric mucosa.

(55) (*Fig. 11*). It was demonstrated for the first time (55) that a few short gastric ischemic episodes induced by celiac artery occlusion results in the gastric protection from the gastric damage induced by prolonged ischemia-reperfusion *via* combining mechanism involving endogenous prostaglandins (PG) derived from COX-1 and COX-2, nitric oxide (NO) mostly due to the overexpression of iNOS and adenosine acting on A_1 receptors (*Fig. 12*). Moreover, mRNA for COX-2 and COX-2 protein were upregulated in the preconditioned gastric mucosa while mRNA and protein expression for COX-1 remained unchanged (55). Furthermore, we have shown that preconditioning of the remote organs to the stomach such as heart or liver by brief episodes of ischemia, that by itself failed to cause gastric damage and produced a small rise in gastric blood flow, exerts a potent protective influence on gastric mucosa subjected to prolonged ischemia-reperfusion (56). To our knowledge, it was the first demonstration of the gastroprotection phenomenon against ischemia-reperfusion by brief ischemic preconditioning of extra-gastric organs (56). Moreover, we confirmed our previous observations that ischemic preconditioning which has been originally described in various organs including heart, lungs, liver, pancreas and intestine,

could be considered as a powerful intervention in the stomach resulting in a remarkable attenuation of the extent of mucosal damage evoked by the severe ischemia-reperfusion (55-57). We assumed that remote preconditioning, affording gastroprotection, involves crucial mediators including PG derived mainly enhanced COX-2 activity and excessive release of neuropeptides from sensory nerves playing a key role in the mechanism of this protection probably due to rise in the GBF resulting in vasodilatation (*Fig. 13*). This notion is supported by our finding that gastroprotection and accompanying rise in the GBF induced by gastric, cardiac or hepatic preconditioning were significantly attenuated by non-selective (indomethacin) and selective COX-1 (SC-560) and COX-2 (rofecoxib) inhibitors (56,57) and by capsaicin ablating functionally sensory nerves that are known to release NO and various vasodilatatory neuropeptides such as CGRP (58-62). Moreover, the concurrent treatment with synthetic PGE_2 analog to compensate for the deficiency of endogenous
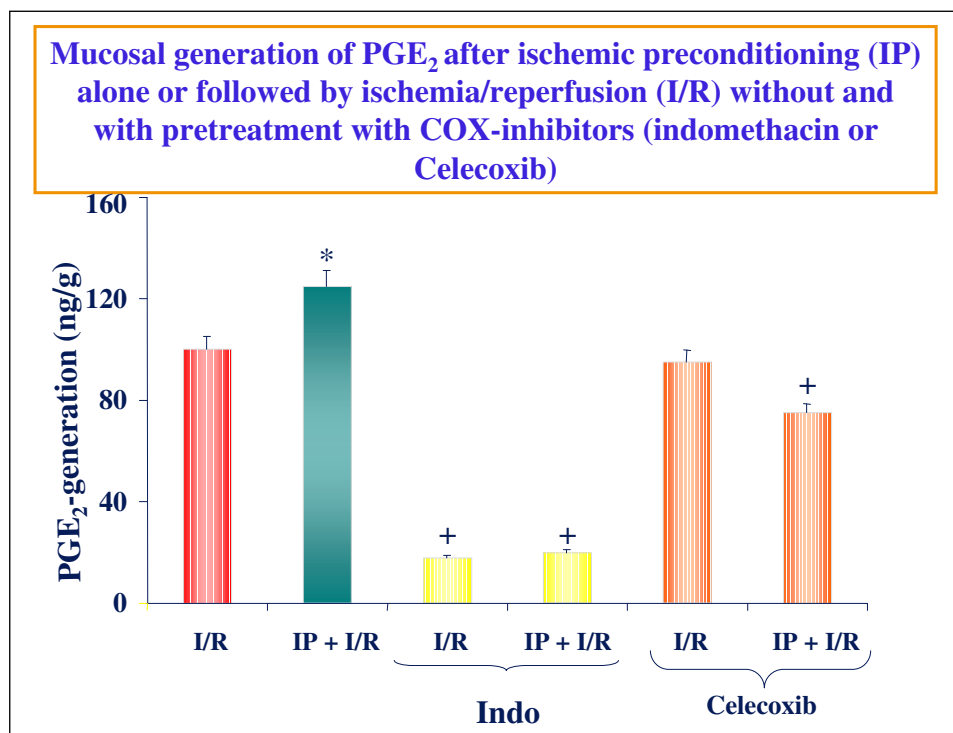
Fig. 12. The generation of PGE_2 in the gastric mucosa subjected to ischemic preconditioning (IP; 2 times 5 min episodes of short ischemia) followed by 30 min of ischemia and 3 h of reperfusion (I/R) with or without pretreatment with indomethacin (5 mg/kg i.p.) or celecoxib (10 mg/kg i.p.). Mean ± SEM of 6 determinations. Asterisk indicates a significant change as compared to the value obtained in animals exposed to I/R. Cross indicates a significant value as compared to the value obtained in gastric mucosa without COX inhibitors.

prostaglandin, or with exogenous CGRP to replace the neuropeptide lost by deactivation with neurotoxic dose of capsaicin of afferent nerves counteracted the deleterious effects of COX-1 and COX-2 inhibitors and capsaicin-induced denervation in preconditioned gastric mucosa exposed to subsequent ischemia-reperfusion (55-57) (*Fig. 14*). Thus, we conclude that PG and many other mediators such as NO, CGRP and polyamines, play an important role in the maintenance of gastric mucosal integrity and in the mechanism of ischemic preconditioning, gastroprotection and gastric adaptation to repeated insults, especially by stress, while gastric adaptation to ASA appears to be PG-independent but probably related to protective growth factors.

In conclusion, endogenous gastric mucosal PG generated by COX-1 and COX-2 play crucial role in adaptive and ischemic gastroprotection activated by mild irritants, growth factors, flavonoids, certain gut hormones but other mediators, especially NO and CGRP released from activated sensory nerves may also contribute to these phenomena (63). Gastric adaptation to repeated NSAID
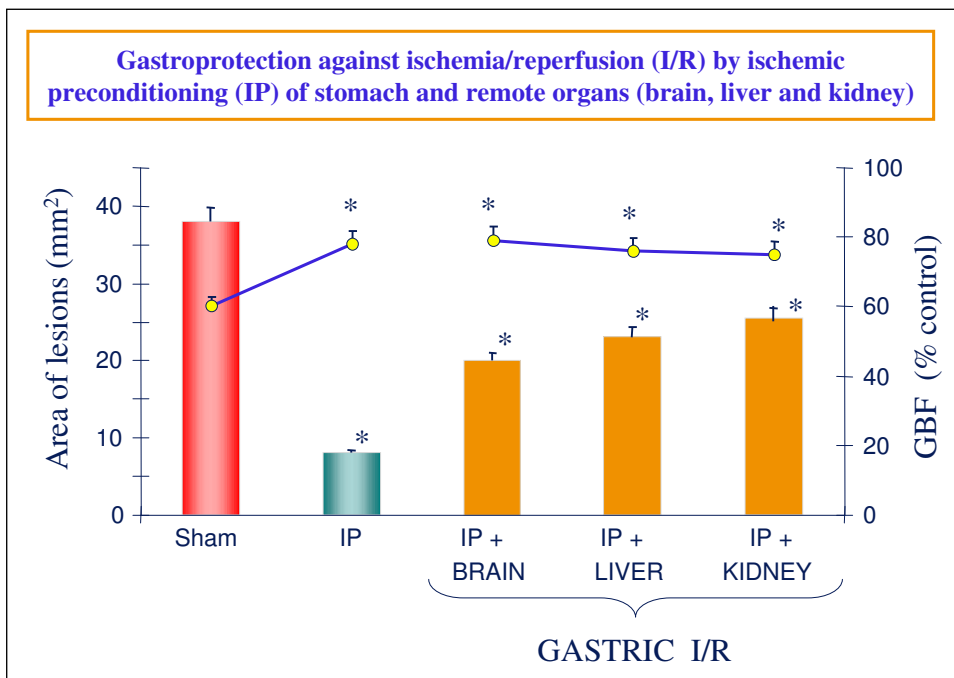
Fig. 13. The effect of short ischemic preconditioning of the stomach (clamping of the celiac artery twice for 5 min) and remote organs such as brain, liver and kidney (2 times 5 min episodes of short ischemia) on the area of gastric lesions and accompanying changes in the gastric blood flow (GBF) in rats induced by prolonged I/R (30 min of ischemia plus 3 h of reperfusion). Ischemic preconditioning significantly attenuates the lesions induced by I/R in the stomach and remote organs, especially, brain and liver. Mean ± SEM of 6-8 rats. Asterisk indicates a significant change as compared to the value obtained in sham-treated animals exposed to prolonged I/R.
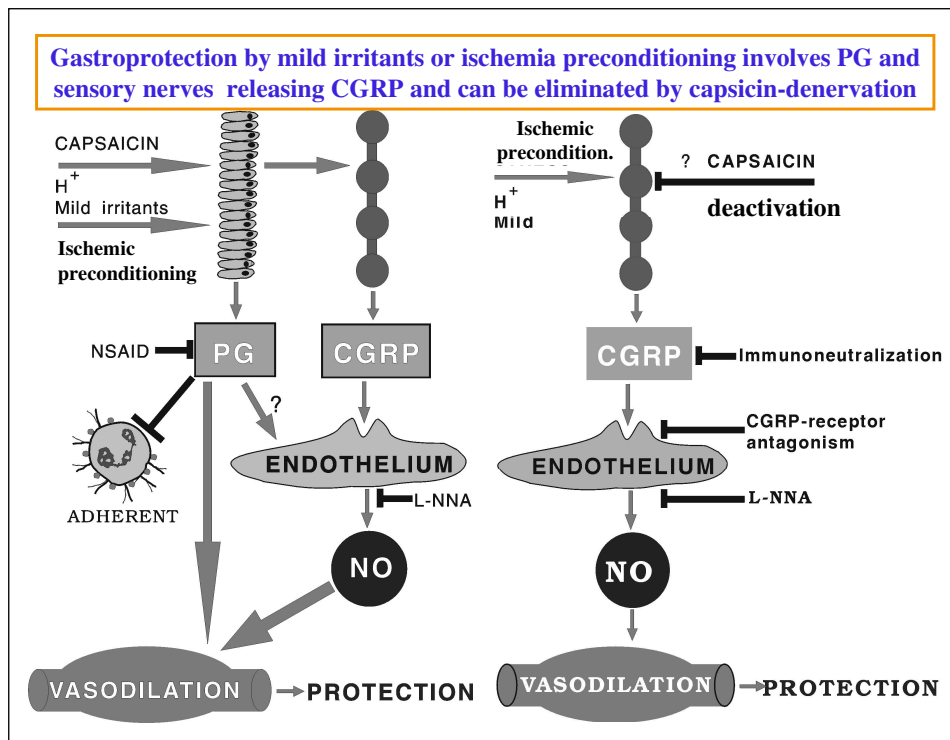
Fig. 14. Schematic representation of the common neural pathways consisting of neuropeptides such as calcitonin gene related peptide (CGRP) released from sensory afferent nerves, endothelium derived nitric oxide (NO) and prostaglandins (PG) from to the gastroprotection and hyperemia w (vasodilatation) induced by mild irritants, low dose of capsaicin and ischemic preconditioning.

application does not depend upon PG in animals and humans. In both, experimantal and clinical settings, the gastric mucosa exposed to ASA or other NSAID showed increased tolerance to repetitive NSAID treatment under the conditions where the PG generation was almost completely suppressed but probably the release of growth factors and NO are involved and accompanied by increased blood flow in NSAID-adapted stomach, an effect that could be reversed by NO-synthase inhibitor.

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