In rats, central vagal stimulation by thyrotropin-releasing hormone protects against ethanol-induced gastric damage by muscarinic release of prostaglandins. In contrast, gastroprotection following capsaicin-induced stimulation of afferent neurons is prostaglandin-independent. Capsaicin-evoked protection is abolished by blockade of calcitonin gene-related peptide (CGRP) receptors and inhibition of nitric oxide (NO) synthase. Various peptides including gastrin 17, cholecystokinin octapeptide, thyrotropin-releasing hormone, bombesin, corticotropin-releasing factor, epidermal growth factor, peptide YY, neuropeptide A analogs and intragastric peptone exert gastroprotection that is abolished by afferent nerve denervation, blockade of CGRP receptors and inhibition of NO synthase. Indomethacin attenuates the protection of some peptides but has no effect with others. The hyperemic response to peptides is mediated by the afferent nerve/CGRP/NO system without contribution of prostaglandins. Furthermore, it was shown that NKA analogs exert afferent nerve-, CGRP- and NO-dependent gastroprotection in the face of substantial reduction of gastric mucosal blood flow indicating that gastroprotection is not necessarily mediated by mucosal hyperemia. In the rat stomach with functioning afferent nerves neither selective inhibition of cyclooxygenase (COX)-1 nor COX-2 is ulcerogenic and only simultaneous inhibition of both COX isoenzymes induces mucosal lesions. In the face of pending injury such as intragastric acid a COX-1 inhibitor evokes dose-dependent damage whereas COX-2 inhibitors are not injurious as long as the function of afferent nerves is not impaired. After afferent nerve denervation, however, COX-2 inhibitors or dexamethasone which suppresses the acid-induced up-regulation of COX-2 are highly ulcerogenic. In conclusion, release of prostaglandins following nerve stimulation can mediate protective effects under certain conditions but is not a prerequisite for neurally mediated mucosal defense. Prostaglandins are of particular importance for the maintenance of gastric mucosal integrity when neuronal defense mechanisms are impaired.

Key words: prostaglandin, afferent sensory nerves, vagus, capsaicin, calcitonin gene-related peptide, nitric oxide, gastric mucosal blood flow, cyclooxygenase-1, cyclooxygenase-2.

The gastric mucosa is continuously exposed to potentially noxious agents. The maintenance of mucosal integrity is ensured by a complex system of
interacting mediators among which prostaglandins play an essential role. During recent years it was shown that neurons constitute an important factor in mucosal defense. From these findings the question arises, whether nerve activation influences the release of protective mediators such as prostaglandins.

**PROSTAGLANDINS AND VAGAL STIMULATION**

Electrical stimulation of the gastric branch of the vagus nerve increases the release of prostaglandins from the isolated, *in vitro* perfused rat stomach and the increase is dependent on the rate of stimulation (1, 2). Hyoscine completely abolishes the effect of parasympathetic nerve stimulation. Chromatographic characterization and quantification of the individual prostaglandins in the perfusates showed that PGE$_2$ and PGF$_{2\alpha}$ are the major prostaglandins released during vagal nerve stimulation. Stimulation of periarterial postganglionic sympathetic nerve fibres does not increase gastric output of prostaglandins (1).

Thyrotropin-releasing hormone (TRH) plays a stimulatory role in the central vagal regulation of gastric functions. High concentrations of TRH receptors and TRH-like immunoreactive fibres and terminals arising from the nucleus raphe pallidus and obscurus are located in the dorsal vagal complex including the dorsal motor nucleus of the vagus and the nucleus of the solitary tract (3—5). TRH nerve terminals synapse on dendrites of gastric vagal motoneurons in rats (5). In addition, electrophysiological studies have shown that TRH increases the efferent activity of the gastric branch of the vagus (6). Intracysternal injection of the stable TRH analog RX 77368 dose-dependently increases gastric PGE$_2$ levels measured in the perfusate of dialysis fibres implanted into the corpus submucosa (7, 8). The stimulatory action of RX 77368 on gastric PGE$_2$ output is blocked by indomethacin and bilateral cervical vagotomy (7). Simultaneously, intracysternal injection of RX 77368 inhibits macroscopic gastric damage induced by ethanol. The protective effect of the TRH analog is completely abolished by indomethacin and atropine (8). These findings indicate that the resistance of the gastric mucosa against ethanol-induced damage following central vagal stimulation is mediated by muscarinic release of PGE$_2$.

**PROSTAGLANDINS AND AFFERENT SENSORY NERVES**

During recent years, extrinsic afferent nerve fibres have been found to play an important role in the resistance of the gastric mucosa against damage. The extrinsic afferent nerves that supply the stomach arise from two different sources. The spinal sensory neurons have their cell bodies in the dorsal root ganglia and reach the stomach via the splanchnic and mesenteric nerves. The afferent fibres in the vagus nerve originate from cell bodies in the nodose and
jugular ganglia. Afferent nerve fibres are particularly abundant around the arterial and arteriolar vasculature in the gastric mucosa. The spinal afferent neurons contain bioactive peptides including calcitonin gene-related peptide (CGRP) and the tachykinins substance P (SP) and neurokinin (NK) A. Extrinsic afferent neurons are unmyelinated or thinly myelinated nerve fibres that are selectively activated by capsaicin. Whereas capsaicin at a low dose stimulates the activity of afferent nerve fibres, high doses cause afferent nerve degeneration in neonatal rats or long-lasting defunctionalization in adult rats (for rev. see 9).

Capsaicin-induced stimulation of afferent neurons enhances gastric mucosal resistance to injury. Thus, intragastric administration of capsaicin prevents gastric mucosal damage evoked by a wide spectrum of injurious factors including ethanol, hydrochloric acid, acidified taurocholate, aspirin and indomethacin (for rev. see 9). In contrast to vagally-mediated gastroprotective effects, protection following stimulation of afferent sensory neurons by capsaicin is independent of the gastric prostaglandin system. Thus, it was shown that intragastric treatment with capsaicin does not increase the release of PGE2 and 6-keto-PGF1α from the rat stomach. Furthermore, the protective effect of capsaicin against challenge with ethanol is not diminished by pretreatment with indomethacin (10). A prominent part of the afferent nerve supply of the stomach contains peptides including CGRP (11). In the isolated vascularly perfused rat stomach, stimulation of afferent neurons by capsaicin releases CGRP into the venous effluent (12) whereas the neonatal treatment of rats with a neurotoxic dose of capsaicin known to cause degeneration of afferent neurons reduces concentrations of CGRP immunoreactivity in gastric mucosal extracts by >96% (11). CGRP8-37, a fragment of human CGRP that lacks the cyclic loop at the aminoterminal of native CGRP, blocks CGRP1-receptors and antagonizes effects of exogenous CGRP in various experimental settings (13). Local close-arterial infusion of CGRP8-37 inhibits the protection conferred by capsaicin against ethanol-induced gastric damage in a dose-dependent manner (14) suggesting that release of CGRP following afferent nerve stimulation mediates the protective effect. The dose of CGRP8-37 that inhibits capsaicin-induced protection corresponds with the dose that antagonizes the protective effect of exogenous CGRP supporting the proposal that release of CGRP from afferent nerves and activation of CGRP1 receptors is crucially involved in the protection evoked by capsaicin (14). Additional evidence for a mediator function of CGRP comes from the finding that administration of anti-CGRP antibodies attenuates protection against ethanol-induced gross and histological mucosal damage conferred by capsaicin. Thus, pretreatment with globulin fractions prepared from serum of a rabbit immunized with rat αCGRP inhibits the protective effect of close arterial infusion of exogenous CGRP as well as of intragastric administration of capsaicin. Globulin fractions prepared from serum of a nonimmunized rabbit or from a serum sample obtained before immunization have no effect (14).
Likewise, a monoclonal antibody to CGRP abolishes capsaicin-evoked gastroprotection (15).

CGRP does not seem to be the final mediator of the protective activity of afferent nerve stimulation. Thus, inhibition of nitric oxide (NO) synthase by N\textsuperscript{G}-nitro-L-arginine (L-NNA) completely blocks the protection conferred not only by capsaicin (16) but also by CGRP (14). The inhibitory effect of L-NNA on capsaicin- and CGRP-induced protection is reversed by co-administration of the substrate of NO synthase L-arginine but not by the inactive enantiomer D-arginine. Similar to the protection following afferent nerve stimulation, the protection elicited by infusion of CGRP is independent of the prostaglandin system. Thus, infusion of CGRP does not increase the release of 6-keto-PGF\textsubscript{1\alpha} from the rat stomach and indomethacin at a dose that virtually abolishes gastric prostaglandin generation does not interfere with the protective effect of CGRP (14).

These findings show that both prostaglandins and NO contribute to the protection following nerve stimulation. Prostaglandins and NO can act as protective mediators either alone or in concert.

PEPTIDE-EVOKED GASTROPROTECTION

A variety of peptides was found to exert potent gastroprotective effects. Thus, in rats gastrin 17 inhibits gastric mucosal damage induced by absolute ethanol in a dose-dependent manner. As shown in Fig. 1 the protective effect is partially inhibited by pretreatment with indomethacin and fully blocked by defunctionalization of afferent nerves evoked by a high, neurotoxic dose of capsaicin, infusion of the CGRP receptor antagonist CGRP\textsubscript{8-37} and injection of anti-CGRP antibodies but not by injection of serum obtained from a nonimmunized rabbit. Furthermore, inhibition of NO synthase by L-NNA abolishes the protection conferred by gastrin 17. L-Arginine but not D-arginine reverses the effect of L-NNA. Effects on gross damage are paralleled by histology (17).

Later on, a number of other peptides administered centrally or intravenously were found to protect the rat stomach against ethanol-induced damage by a similar mechanism including TRH, cholecystokinin octapeptide (CCK-8), bombesin, corticotropin-releasing factor (CRF), epidermal growth factor (EGF), peptide YY (PYY) and NK\textsubscript{2} receptor agonists (Table 1). The protective effect of all these peptides depends on afferent neurons and is abolished by afferent nerve denervation, infusion of CGRP\textsubscript{8-37} and inhibition of NO synthase. The contribution of prostaglandins to the protective activity differs between the various peptides. Thus, indomethacin completely antagonizes the protective effect of TRH and partially attenuates the protection conferred by gastrin 17,
CCK-8, bombesin and EGF, whereas the protection induced by PYY and the NK2 receptor agonists is not or to a negligible extent affected by indomethacin (Table 1). These findings suggest that prostaglandins are involved in the protective activity of some but not all peptides.

A gastroprotective effect is also observed when the gastric lumen of rats is perfused with peptone solution (30). The protection conferred by peptone is near-maximally inhibited by gastrin immunoneutralization, inactivation of capsaicin-sensitive afferent neurons, CGRP immunoneutralization, blockade of gastrin (CCK-B), CGRP, bombesin/gastrin-releasing peptide (GRP), or somatostatin receptors, and by the NO synthase inhibitor NG-nitro-L-arginine methyl ester (L-NAME). Peptone-evoked protection is partially by 46% counteracted by atropine and to a comparable extent by methylatropine indicating the involvement of peripheral muscarinic receptors. Indomethacin and the selective cyclooxygenase (COX)-2 inhibitors NS-398 and L-745,337 partially antagonize the peptone-induced protection in a dose-dependent manner. Dexamethasone is ineffective indicating that prostaglandins derived from a constitutive COX-2 but not from an induced enzyme contribute to the protective effect of peptone (30). Whether prostaglandins generated via the COX-1 pathway are also involved has not been elucidated so far. These results show that the gastroprotection conferred by peptone is mediated by endogenous gastrin and possibly somatostatin and relies on capsaicin-sensitive afferent,

<table>
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Table 1. Role of prostaglandins, afferent neurons, CGRP and NO in peptide-induced gastroprotection
cholinergic and bombesin/GRP neurons. In vitro incubation experiments of rat antrum mucosa have shown that intraluminal peptone stimulates release of CGRP demonstrating activation of afferent neurons (31). Again, the final protective mediator seems to be NO as L-NAME abolishes the protective activity of peptone and the effect is reversed by L-arginine (30). A mediator function of afferent neurons has also been demonstrated for other peptone effects. Thus, the gastric acid secretory responses induced by peptone involve capsaicin-sensitive afferent neurons (32). In vivo and in vitro studies in various species have shown that peptone and other protein instillates stimulate gastrin secretion (33). Circulating gastrin levels reached during perfusion of the gastric lumen with peptone are in the same order of magnitude as those measured after injection of a protective dose of exogenous gastrin (17). This could imply that endogenously released gastrin mediates the protective effect of peptone. This proposal is substantiated by the finding that gastrin immunoneutralization or blockade of gastrin (CCK-B), but not CCK-A receptors, inhibit the protective effect of intraluminal peptone (18, 30).

Using the vascularly perfused rat stomach, it was shown that peptone stimulates gastrin secretion by activating intramural cholinergic and noncholinergic bombesin/GRP neurons (34). This was concluded from the fact

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**Fig. 1.** Gastroprotective effect of gastrin 17. Rats were treated with a bolus i.v. injection of gastrin 17 (25 pmol/kg) 10 min before oral instillation of 1 ml of absolute ethanol. Additional rats were pretreated with indomethacin (20 mg/kg, p.o.), the CGRP$_1$ receptor antagonist CGRP$_{8-37}$ (500 pmol/kg/min for 20 min, i.v.) anti-CGRP antibodies (i.v.), the NO synthase blocker L-NNA (3 mg/kg, i.v.) or L-arginine (100 mg/kg, i.v.) plus L-NNA. Gastric damage was assessed by calculation of a lesion index 5 min after ethanol instillation. Values are mean ± SEM of 6—9 rats. *P<0.001 vs controls; †P<0.001 vs gastrin alone. Data are modified from Stroff et al. (17).
that the peptone-evoked gastrin release was fully blocked by tetrodotoxin and partially inhibited by atropine or a selective bombesin/GRP receptor antagonist (34). The reversal of the peptone-induced protection by atropine, methylatropine and the selective bombesin/GRP receptor antagonist D-22213 could thus result from interference with the peptone-evoked release of gastrin or, alternatively, from involvement of bombesin/GRP receptors in the protective activity of endogenous gastrin (30). In addition, bombesin exerts gastroprotective effects (22) that are at least partially mediated by release of gastrin (23).

Most protective peptides are potent vasodilators. Thus, in rats hyperemic effects have been demonstrated e.g. for TRH (35), gastrin (18), CCK-8 (36) and bombesin (Fig. 2) (37). The increase in gastric mucosal blood flow elicited by the protective peptides involves cholinergic pathways and activation of muscarinic receptors, stimulation of afferent neurons, release of CGRP and formation of NO as final mediator of the vasodilation. Indomethacin attenuates the protective activity of TRH (8), gastrin (Fig. 1) and bombesin (23) but has no effect on the hyperemia evoked by protective peptides (36). An increase in gastric mucosal blood flow has been suggested to play an important role in the resistance of the mucosa against injury (9) and may contribute to the protective effect of gut peptides. However, the finding that indomethacin is involved in the protection induced by certain gut peptides but has no effect on their hyperemic response indicates that at least the prostaglandin-dependent part of gastroprotection is not mediated by increased gastric mucosal blood flow but results from effects not involving vasodilation.

Even gastroprotection that relies on stimulation of afferent nerves is not necessarily mediated by or associated with increased gastric mucosal blood flow. The tachykinin analogs NKA-(4-10) and [Ala$^5$,β-Ala$^8$]NKA-(4-10) specifically stimulate NK$_2$ receptors (38). In rats, intravenous injection of NKA-(4-10) and [Ala$^5$,β-Ala$^8$]NKA-(4-10) confers dose-dependent protection against damage induced by 50% or absolute ethanol or acidified taurocholate (28, 29). The protective effect is completely lost in rats treated with a neurotoxic dose of capsaicin to induce afferent nerve denervation. As shown previously for other afferent nerve-dependent protective effects the protection conferred by the NKA analogs is also blocked by infusion of the CGRP receptor antagonist CGRP$_{8-37}$ and inhibition of NO synthase by L-NAME. The protective effect of the NKA analogs is receptor-mediated and antagonized by the NK$_2$ receptor antagonist MEN-10,627 but not by the NK$_1$ receptor antagonist SR-140333 (29). These findings indicate that activation of NK$_2$ receptors protects against various forms of gastric damage by a mechanism depending on activation of afferent neurons. The NKA analogs do not affect acid secretion (29). The protective effect of NKA analogs can, therefore, not be explained by acid back-diffusion following an elevated gastric acid output but
seems to result from a direct stimulatory effect on afferent neurons. This suggestion is in keeping with the finding derived from functional studies that tachykinin receptors exist on primary afferent nerves in the rat bladder wall (39).

Although the NKA analogs exert protection by a mechanism involving stimulation of afferent neurons, they do not increase gastric mucosal blood flow. In contrast to other protective peptides such as bombesin, a marked, near-maximal drop in gastric mucosal blood flow occurs after administration of the NK₂ receptor stimulating agents simultaneous with full protective activity (Fig. 2). Obviously, the potent direct vasoconstrictor action of NK₂ receptor activation on gastric mucosal/submucosal vessels and the systemic hypotensive action of the tachyklinins overcome the local vasodilating effect of CGRP and NO. These observations indicate that vasodilation and gastric mucosal hyperemia are not a prerequisite for protective effects conferred by activation of primary afferent neurons.

Tachykinins such as SP and NKA have been localized in capsaicin-sensitive afferent nerves (38). Furthermore, in various organs, tachyklinins are colocalized with CGRP in the same neuronal structures (38). In the rat stomach, administration of capsaicin is found to promote release of CGRP and NKA (40). As shown in Fig. 3, blockade of NK₂ receptors by MEN-10,627
partially by 65% inhibits the increase in gastric mucosal resistance evoked by intraluminal capsaicin suggesting that CGRP and NKA act as cotransmitters to mediate protective effects of afferent nerve stimulation (29). Activation of NK₁ receptors does not seem to contribute to afferent nerve-mediated protection (29). In contrast to the protective effect, the hyperemic response to capsaicin is not attenuated by NK₂ receptor blockade (Fig. 3). This observation is further support of the proposal that gastric mucosal hyperemia is not the primary or exclusive mechanism of afferent nerve-mediated protection.

**ROLE OF CYCLOOXYGENASE ISOENZYMES IN RATS WITH INTACT AND DENERVATED AFFERENT NEURONS**

Although prostaglandins do not mediate the protection following capsaicin-induced afferent nerve stimulation, their contribution to mucosal defense is influenced by the activity of the afferent neural system.
Prostaglandins are biosynthesized from arachidonic acid by the activity of the enzyme cyclooxygenase (COX). Two isoforms of cyclooxygenase, COX-1 and COX-2, have been identified. COX-1 is expressed constitutively and high levels can be detected in most tissues (41). Levels of COX-2 mRNA and protein are usually low or undetectable under basal conditions but are rapidly elevated during inflammation or mitogenic stimulation (42, 43). It was postulated that COX-1-derived prostaglandins mediate homeostasis reactions, in particular the resistance of the gastric mucosa against injury, whereas COX-2-derived prostaglandins are involved in pathological reactions such as inflammation (44). Recent studies, however, revealed that COX-2 also has an important role in the maintenance of gastric mucosal integrity. Thus, in rats selective inhibition of COX-2 markedly aggravates gastric damage following ischemia-reperfusion (45), antagonizes the gastroprotection conferred by the mild irritant 20% ethanol (46) or luminal perfusion with peptone (30) and delays the healing of chronic gastric ulcers (47, 48). In the normal rat stomach with intact afferent neurons and in the absence of a potentially noxious agent in the gastric lumen, neither selective inhibition of COX-1 by SC-560 nor of COX-2 by rofecoxib is ulcerogenic. Severe lesions, however, develop after simultaneous administration of a COX-1 and COX-2 inhibitor (49, 50). In contrast, when the gastric mucosa is challenged with 0.3 N HCl selective inhibition of COX-1 by SC-560 is sufficient to induce dose-dependent gastric mucosal injury. Even in the presence of intragastric acid selective inhibition of COX-2 by rofecoxib or DFU is not ulcerogenic as long as the function of afferent neurons is preserved (49). However, when rats are pretreated with a neurotoxic dose of capsaicin to denervate afferent nerves, the selective COX-2 inhibitors DFU and NS-398 induce dose-dependent and severe mucosal injury. A similar induction of mucosal damage in the acid-challenged stomach after afferent nerve denervation has previously been shown for the non-selective COX inhibitor indomethacin (52). Intragastric instillation of acid increases the expression of COX-2 mRNA and this effect is inhibited by dexamethasone whereas gastric mucosal levels of COX-1 mRNA are not changed (49). Similar to the inhibition of COX-2 enzyme activity by DFU or NS-398, suppression of the acid-induced up-regulation of COX-2 by dexamethasone induces severe gastric mucosal injury in afferent denervated rats but not in rats with intact afferent neurons further supporting the essential role of COX-2-derived prostaglandins in mucosal defense after afferent nerve denervation (51).

CONCLUSIONS

Central or peripheral vagal stimulation releases prostaglandins from the rat stomach. This effect can contribute to the gastroprotection following vagal
stimulation. Prostaglandin release, however, is not a prerequisite for neurally mediated mucosal defense. Thus, the protection conferred by stimulation of afferent neurons is not associated with increased release of gastric prostaglandins and is not diminished by indomethacin. Protection following afferent nerve stimulation is abolished by CGRP1 receptor blockade or CGRP immunoneutralization and inhibition of NO synthase. Various peptides exert potent protective effects that are mediated by afferent nerve stimulation, CGRP and NO. In addition, prostaglandins contribute to the protective activity of some but not all peptides. The role of gastric mucosal hyperemia in neurally mediated gastroprotection is uncertain as the prostaglandin-dependent part of peptide-induced protection does not rely on gastric mucosal hyperemia. Furthermore, afferent nerve-mediated gastroprotection, e.g. by NK2 receptor agonists can occur in the face of a marked decrease in gastric mucosal blood flow. Finally, it has been shown that formation of prostaglandins is of particular importance for the maintenance of gastric mucosal integrity when neuronal defense mechanisms such as sensory afferent nerves are impaired.

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Received: October 3, 2001
Accepted: October 18, 2001

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