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The Role of Capsaicin-Sensitive Afferent Nerves in Gastric Mucosal Protection Initiated Centrally or Peripherally under Experimental Conditions

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1. Introduction

How can gastric mucosa resist to wide variety of endogenous (e.g. acid, pepsin, *H. pylori*) and exogenous (bacterial products, NSAIDs, alcohol, cytotoxic agents) mucosal challenges? Or how can it be explained that while the incidence of mucosal injury related to acid secretion is about 20% of the population, everybody secretes acid throughout the life [1]? And how can be answered the classic question of Davenport: why the stomach does not digest itself [2]?

Though accumulating data strongly suggest an apparent redundancy in the processes of gastric mucosal defense, several mediators, mechanisms, structural elements involved in mucosal protection have been identified, the precious mechanism has not been completely clarified yet and incompletely understood.

The aim of present work is to give a short overview on the protective mechanism of gastric mucosal protection, particularly on capsaicin sensitive afferent pathways and mediators released from sensory nerve endings and play a role in maintaining gastric mucosal integrity.

2. Gastric mucosal defense: mechanisms, mediators

Gastric mucosal defense to acid and other noxious stimulus involves both structural and functional components. Gastric mucosal barrier consists of the pre-epithelial mucus bicarbonate layer, an epithelial layer, and a post-epithelial layer. The pre-epithelial mucus bicarbonate layer consist of mucus gel, bicarbonat surfactant phospholipids, treofil peptides. Epithelial layer, and a post-epithelial layer involve blood vessels, non-epithelial cells and enteric nerves

play a role in generation of different substances which are essential in maintaining mucosal integrity and gastric mucosal defense, e.g. bicarbonate, mucus, phospholipids, trefoil peptides, prostaglandins (PGs), heat shock proteins [reviews 3-5].

2.1. Mucosal barrier

The pre-epithelial layer protects the gastric mucosa from microscopic damage. This layer consists of mucus gel, bicarbonate and surfactant phospholipids, which cover the mucosal surface. It retains bicarbonate secreted by surface epithelial cells to maintain a neutral micro-environment [5-7].

Trefoil factor family (TFF) proteins are able to enhance mucosal barrier functions by stabilizing the mucus gel and promoting epithelial restitution [8, 9]. These small protease-resistant proteins have been demonstrated to be protective demonstrated in various ulcer models [10, 11], the exact molecular mechanism has not been clarified yet.

2.2. Prostaglandin

Though the term „cytoprotection“ has been used first by Chaudhury and Jacobson [12] the classic definition of cytoprotection derives from Robert et al [13]; they suggested that cytoprotection refers to protection of gastric mucosa against chemically/ physically-induced acute gastric ulceration in doses much smaller than that reduce gastric acid secretion. According to their results prostaglandins fulfilled this criteria. Later Lacy and Ito [14] reported that the superficial layer, that is supposed to be highly involved in maintenance of mucosal barrier, is not preserved during prostaglandin-induced protective processes; this was the main argue against the term “cytoprotection”.

Prostaglandins are synthesized from arachidonic acid by all nucleated cells, but the highest concentrations are found in gastrointestinal mucosa. Continuous synthesis of prostaglandins by gastrointestinal mucosa most likely represents a physiological process necessary to maintain cellular integrity and for this process prostaglandins serve as trophic factors [15, 16]. Subsequent studies proved that the maintenance of gastric mucosal blood flow and thus the prevention of ischemia are the key elements in the mucosal protective effect of prostaglandins [17]. Takeuchi et al. [18] suggested recently, that endogenous PGs also contribute to maintaining mucosal integrity after barrier disruption through an increase in mucosal blood flow, which occurs via sensory neurons by activation of the EP₁ receptor.

Inhibition of leukocyte adherence to the vascular endothelium and inhibition of apoptosis are additional actions that can result in prevention of gastric mucosal damage [19].

Different prostaglandin receptors are involved in different processes resulting in mucosal defense. The effects of PGE₂ on various gastric functions are mediated by different EP receptor subtypes; such as inhibition of acid secretion (EP₃) and motility (EP₁), stimulation of mucus secretion (EP₄) and HCO₃⁻ secretion (EP₁), and an increase in mucosal blood flow (EP₂/EP₄). However, the presence of EP₁ receptors is essential to the protective action of PGE₂ [20].

2.3. Nitric oxide

Nitric oxide (NO) is a freely diffusible molecule, synthesized from L-arginine by the enzyme NO synthase, exerts its biological action on vascular smooth muscle by stimulating soluble guanylate-cyclase and the subsequent formation of cyclic guanosine-monophosphate, which results in vasodilation.

NO has been shown to participate in the regulation of gastric mucosal microcirculation both under resting and stimulated conditions, since inhibition of NO synthase significantly reduced both the resting and the gastric acid-induced increased gastric mucosal blood flow [21, 22]. In addition, inhibition of NO synthase increases gastric mucosal injury [23, 24], and the constitutive isoform of NO synthase is important in maintaining gastric mucosal integrity [25].

NO mediates the mucosal vasodilatation and gastroprotective effect of calcitonin gene-related peptide (CGRP), which is released from nerve terminals of primary sensory neurons and plays a crucial role in gastric emergency system [26-28].

2.4. Protein and non-protein sulfhydryls

Protein and non-protein sulfhydryls were suggested also as endogenous protective compounds [29, 30], and it was raised that the maintenance of a critical level of non-protein sulfhydryls in the gastric mucosa is important for the gastroprotective action - besides nitric oxide [31].

2.5. Hydrogen sulfid

H₂S like NO, is an important gaseous mediator of gastric mucosal protection [32], and inhibition of endogenous H₂S synthesis increases the susceptibility of the gastric mucosa to the damaging action of non-steroidal anti-inflammatory drugs (NSAIDs) [33]. On the other hand, exogenous H₂S donors can increase the resistance of the gastric mucosa to injury induced by NSAIDs [34].

The gastroprotective effect of H₂S involves several mechanisms, such as maintenance of gastric mucosal blood flow, stimulation of bicarbonate secretion, inhibition of pro-inflammatory cytokine expression/release stimulation of prostaglandin synthesis, reduced leukocyte-endothelial adherence, decreased reactive oxygen metabolite production and enhanced tissue repair [35].

2.6. Matrix metalloproteinases

Matrix metalloproteinases (MMPs) play a role both in the pathogenesis and in healing of peptic ulcers. These endopeptidases degrade extracellular matrix proteins and are essential for extracellular matrix remodeling and wound healing [36]. Experimentally their altered expression have been demonstrated in gastric ulcers induced by NSAIDs (indomethacin, naproxen) or ethanol [37, 38].

3. Activation of gastric mucosal protective processes by noxious stimulus

Noxious stimulus results in both generation of endogenous substance(s) that may counteract or attenuate the mucosal injury, and initiation of further protective mechanisms or formation of additional protective mediators that stimulate repair and restore mucosal integrity.

3.1. Hypoxia-inducible factor-1

Early phase of mucosal lesions is characterized by decrease of mucosal blood flow and the consequent hypoxia. However, activation of hypoxia-inducible factor-1, in response to hypoxia, induces trefoil factor family (TFF) gene expression in rat and human gastric epithelial cells. The consequent production of TFF peptides augment surface mucous barrier functions (see above) and stimulate epithelial restitution by increasing mucosal blood flow, migration of cells and restoration of barrier function [39, 40].

3.2. Antioxidant enzymes

Gastric mucosal injury is characterized by excessive production and/or decreased elimination of reactive oxygen species (ROS), that can induce tissue damage by promoting lipid peroxidation and increasing the production of inflammatory mediators and proinflammatory cytokines. Antioxidant enzymes, such as superoxide dismutase (SOD), catalase or glutathione are able to counteract the oxidative stress, for example, SOD converts superoxide radical anion ($O_2^{\cdot-}$) into less noxious hydrogen peroxide (H_2O_2) [39].

3.3. Heme oxygenase-1

Heme oxygenase-1 (HO-1), the inducible form of heme oxygenase, also exerts cytoprotective effect and its generation may be induced by oxidative stress, inflammatory cytokines or heavy metals [40]. HO-1 catalyzes the oxidative degradation of the pro-oxidant heme to antioxidant and cytoprotective carbon monoxide and biliverdin [41, 42]. The activity of HO-1 is also increased during the healing of gastric ulcers, which indicates its involvement in the mucosal repair processes [43].

3.4. Growth factors

In addition, several genes, encoding peptides, are also upregulated after mucosal damage in the gastrointestinal mucosa and stimulate the repair process. *Epidermal growth factor (EGF)* a potent stimulant of growth and repair when infused systemically. It inhibits gastric acid secretion, and has a cytoprotective effect on the gastrointestinal mucosa. On the other hand, EGF is one of the main peptides secreted by repair lineages of the gastrointestinal tract [44]. *Trefoil factor family (TFF)* has both cytoprotective and stimulant effect on repair. Three members (TFF1/pS2, TFF2/SP, and TFF3/ITF) of the family are present in human gastrointestinal mucosa. They are produced rapidly at sites of injury and stimulate the repair process. Exogenous TFF2/SP exerts a cytoprotective action in indomethacin-induced ulcer model in rats [45]. *Transforming growth factor α (TGF- α)* is normally trophic both in vitro in different cell lines and in vivo

in the intestine of rats [46]. TGF- α decreased the gastric damage induced by ethanol or stress [47], and enhanced immunoreactive TGF- α was measured in gastric juice (within 30 minutes) following gastric instillation of hydrochloric acid. *Transforming growth factor β (TGF- β)* is also involved in mucosal defense, but in contrast to other growth factors, it regulates the epithelial proliferation, it halts the proliferation of epithelial cells once they have left the crypts or glands. In addition, it is also a potent stimulant of cell migration [48-50]). *Hepatocyte growth factor (HGF)* is secreted as inactive precursor and then converted to an active form. HGF receptor was shown to be increased in the gastric mucosa after injury [51]. It was suggested that, the active HGF, produced in the stomach after injury, may stimulate the proliferation of gastric mucosal epithelial cells through increased number of HGF receptor. *Basic fibroblast growth factor (bFGF)* a potent stimulant of angiogenesis, and accelerates the healing of experimental gastric and duodenal ulcers in rats [52, 53]. In ulcerated human gastric mucosa, immunoreactive bFGF is upregulated [54], thought additional data suggest that bFGF mRNA expression is an early event after mucosal injury [55]. *Platelet derived growth factor (PDGF)* also stimulates angiogenesis. It was shown that PDGF (and bFGF) accelerated the healing of cysteamine induced duodenal ulcers in rats by stimulating angiogenesis and granulation tissue formation [56]. Furthermore, in experimental duodenal ulcer and colitis in rats (after a transitory reduction) of the concentrations of bFGF and PDGF increased in the healing phases both experimental lesions [57]. *Vascular endothelial growth factor (VEGF)* has dual role; in acute gastric ulcer it induced gastroprotective effect surprisingly by enhancing vascular permeability and the consequent perivascular dilution barrier towards gastric mucosal irritants [58]. On the other hand, in chronic duodenal ulcer VEGF stimulates granulation tissue formation and angiogenesis resulted in ulcer healing. In addition, upregulation of VEGF mRNA expression was observed after ethanol challenge [59].

3.5. Capsaicin-sensitive primary afferent neurons

When the gastric epithelium is exposed to acid back diffusion or any irritant, one of the most rapid and important responses is an increase in mucosal blood flow. The aim of this hyperemic response is to remove, dilute and buffer any back-diffusing injurious agent or acid. This response is mediated by primary sensory afferent neurons, namely if the mucosal barrier is disturbed or disrupted in the presence of luminal acid, the acid reaching the lamina propria stimulates spinal afferents. This stimulation will initiate the efferent-like function of primary afferents resulting in CGRP and NO formation and release that induces a prompt hyperaemia in the gastro-duodenal mucosa and bicarbonate (HCO_3^-) secretion [60-68].

3.5.1. Capsaicin-sensitive primary afferent neurons and transient receptor potential vanilloid-1 (TRVP1) receptors

Afferent neurons have a basic role in regulation of gastrointestinal functions, and also in gastric mucosal defense. Beside intrinsic sensory neurons which have their cell body within the gastrointestinal tract and originate in the myenteric or submucosal plexus, there are two groups of extrinsic sensory neurons: vagal and spinal afferents. 75-90% of the axons in vagus nerve are afferent fibers [69], and 9% of these afferent fibres are the capsaicin-sensitive afferent

nerves [70], that originate in the jugular and nodose ganglia and project to medullary brain stem. The spinal afferents have their cell body in dorsal root ganglia and reach the stomach via spinal and splanchnic nerves.

The first evidence that suggested the prominent role of sensory nerves in gastric mucosal resistance was obtained by experiments when ablation of sensory nerve was shown to result in aggravation of mucosal lesions induced by hydrochloric acid, aspirin, indomethacin [review 71]. Moreover, Szolcsányi and Barthó described that stimulation of afferent nerves by capsaicin resulted in mucosal protection against acid induced mucosal injury [63, 71]. The role of sensory nerves in gastric mucosal defense was confirmed by the findings that capsaicin induces gastroprotective action following intragastric administration, both in experimental animals [71, 72] and in humans [73].

The protective action of capsaicin is due to its stimulatory effect on *capsaicin-sensitive* primary afferent neurons, since defunctionalisation of afferent neurons by high parenteral dose of capsaicin [74] abolished the gastroprotective effect of capsaicin [67, 71].

The action of capsaicin is mediated by capsaicin receptor which was cloned and named as transient receptor potential vanilloid-1 (TRPV1) [75], the specific sensor for capsaicin [76-79].

In the gastrointestinal tract, TRPV1 can be identified in intrinsic enteric neurons, extrinsic sensory neurons, epithelial and endocrine cells [80-84].

The acid-evoked hyperemia in the esophageal and duodenal mucosa is inhibited by the TRPV1 antagonist capsazepine or sensory denervation, suggesting that acid activates TRPV1 on sensory nerve fibers [85, 86]. As a result, activation of TRPV1-positive sensory nerve fibers are able to protect the esophageal, gastric and intestinal mucosa from a variety of injurious by stimulating mucosal microcirculation [61].

3.5.2. Peptide mediators of capsaicin sensitive primary afferent neurones

Apart from signaling to the CNS, TRPV1-expressing sensory nerve fibers when activated, peptide transmitters, such as CGRP, somatostatin and the tachykinins substance P (SP) and neurokinin, as well as well as nitrogen oxide (NO) and /or ATP can be released from the peripheral nerve endings of afferents nerves and can influence the gastrointestinal function [61, 87-89]. Peptide mediators of TRPV1-positive afferent neurons may induce wide variety of actions.

CGRP and substance P fibers innervate the mucosal and submucosal microvasculature [78, 90]. CGRP in rat stomach are located exclusively in extrinsic afferent fibers, most if not all, in spinal sensory afferent neurons [91-93]. Others also confirmed that CGRP, substance P, somatostatin were characteristic of *capsaicin-sensitive* DRG neurons [77, 78, 93-95], while CGRP was absent from vagal afferent neurons containing TRPV1 [91]. However, Suzuki et al. [96] demonstrated that the density of CGRP-immunoreactive fibers in the mucosa was largely reduced also by vagotomy suggesting CGRP-immunoreactive fibers to be of both vagal and spinal origin. The CGRP-and SP-immunoreactive fibers were less influenced by vagotomy in

the submucosal and muscular layers. Furthermore, major portion of SP-immunoreactive fibers in the mucosa is of vagal origin, and in contrast to CGRP, SP-immunoreactive fibers are not capsaicin sensitive. In contrast, others showed that capsaicin also induces the release of SP from guinea pig and rat stomach [97]. Sternini et al [93] suggested that while CGRP-containing nerve fibers arise exclusively from extrinsic neurons, SP-containing fibers are of both extrinsic and intrinsic origin.

The gastroprotective effect of both CGRP and capsaicin was blocked by CGRP₁ receptor antagonist, CGRP 8-37 [98]. However, not only CGRP₁ receptor may mediate gastroprotective effects, since CGRP₁ receptor antagonist failed to influence the effect of CGRP against aspirin-induced lesions [99]. CGRP/NO released from the nerve terminal of sensory afferents, induces gastroprotective effect not only by increasing mucosal microcirculation, but also by stimulation of mucin synthesis in the gastric corpus mucosa [100] and by reduction of myoelectrical activity in gastric smooth muscle [101].

On the other hand, TRPV1 activation has been found to exacerbate inflammation and tissue injury [76]. In addition, in the murine gastric mucosa, in ethanol-induced injury TRPV1-mediated release of neuronal substance P and subsequent formation of reactive oxygen species are involved in the development of mucosal damage [102]. Moreover, colitis induced by dextran sulfate or trinitrobenzene sulfonic acid in rodents was attenuated by TRPV1 antagonist or TRPV1 knockout animals [103, 104]. TRPV1 receptors may also play a proinflammatory role in the ileitis elicited by *C. difficile* toxin A. Formation of endocannabinoids is supposed to be involved in this process, and endocannabinoids stimulate TRPV1, consequently, induce the release of substance P from sensory nerve fibers. Substance P activates enteric neurons and immune cells, which finally results in hypersecretion, inflammation, and mucosal damage [105].

How can CGRP mediate gastric mucosal protection? As mentioned above the gastric mucosa and submucosa are densely innervated by CGRP, which is localized in capsaicin-sensitive afferent fibers. The protective effect of CGRP was lost after blockade of the nitric oxide system, but not the prostaglandin system indicating that the vasodilator and the consequent gastroprotective effect of CGRP is due to NO, but not to PG release [106]. However, the role of prostaglandins in sensory nerve-mediated gastroprotection was suggested by the findings that indomethacin inhibited the mucosal protective effect of capsaicin [107]. Takeuchi et al [108] concluded that capsaicin exhibits gastric cytoprotection, essentially by stimulating sensory neurons, and this action is facilitated by endogenous prostaglandins through EP₂/IP receptors, probably sensitizing the sensory neurons to capsaicin.

Substance P, released also from afferent nerve fibers, do not mediate vasodilation in the rats stomach, neither the NK₁ or NK₂ antagonists influenced capsaicin-induced vasodilatation nor tachykinins dilated the submucosal arteries and increased gastric mucosal blood flow; on the contrary, substance P and neurokinin A induced constriction of submucosal veins [109, 110] and substance P was supposed to be involved in the pathomechanism of ethanol-induced gastric mucosal damage [111, 112]. However, others reported that substance P induce vasodilatation in vivo [113], and the effect could be attenuated by N^G-monomethyl-L-arginine – a

NO synthase inhibitor – suggesting the role of endogenous NO in the vasodilator response of substance P.

3.5.3. Role of capsaicin sensitive afferent nerves in action of peripherally acting gastroprotective agents

Capsaicin sensitive afferent fibers are also involved in the mucosal protective action of several gastroprotective agents. E.g. defunctionalisation of capsaicin-sensitive afferent nerves by pretreatment with a neurotoxic dose of capsaicin aggravates gastric ulcers in rats and antagonised the gastroprotective effect of capsaicin (10 mg/kg p. os) and lafutidine indicating that gastroprotective activity mediated by capsaicin-sensitive afferent nerves [114].

Similarly, the gastroprotective effect of different flavonoids against ethanol-lesions was reduced by a neurotoxic dose of capsaicin, or by pretreatment with NO synthase inhibitor N^G-nitro-L arginine, indicating that plant-originated flavonoids-induced gastroprotection probably due to enhancement of the expression of constitutive NO synthase and the release of CGRP from sensory afferent nerves [115].

EGF was shown to exert a protective role against ethanol-induced gastric mucosal injury (10-30 µg, intragastrically), possibly by dilating the gastric mucosal arterioles via capsaicin-sensitive afferent neurons involving CGRP and NO mechanisms. Namely, the protective effect of EGF was significantly inhibited by pretreatment with capsaicin desensitization, human CGRP₁ antagonist hCGRP 8-37, or the NO synthesis inhibitor, N^ω-nitro-L-arginine methyl ester [116].

Mozsik et al [117] suggested participation of vanilloid/capsaicin receptors, CGRP and substance P in gastric protection of omeprazole and omeprazole-like compounds.

Moreover, accumulating evidence indicates that capsaicin sensitive afferent fibers play a pivotal role not only acutely in gastroprotection, but also in ulcer healing. In chronic ulcer model, the acetic acid-induced model neurotoxic dose of capsaicin (100 mg/kg s.c.) significantly increased ulcer size, decreased gastric mucosal cell proliferation at the ulcer margin, angiogenesis in the granulation tissue and also gastric mucus content. In addition, the dramatic increase in elevation of EGF levels in salivary glands and serum was completely abolished by neurotoxic dose of capsaicin. The results suggest that capsaicin sensitive nerves contribute to gastroprotective and ulcer healing processes in the stomach probably by stimulation of EGF expression in salivary glands [118].

3.5.4. Role of capsaicin sensitive afferent fibers and in the action of centrally acting gastroprotective agents

Besides the periphery, gastric mucosal integrity can be regulated also by central mechanism. In the last decades increasing number of evidence suggested that central nervous system (CNS) has a pivotal role in regulation of gastric mucosal integrity. Different brain areas have been suggested to be involved in centrally induced gastroprotection. Among them, hypothalamus and dorsal vagal complex (DVC) seem to have a particularly important role and well defined interconnections between neuroendocrine hypothalamus and the central autonomic system

have been described. Descending hypothalamic efferents carry feedback signals to viscerosensory and brainstem catecholaminergic neurons and regulatory inputs to parasympathetic (dorsal vagal nucleus) and sympathetic (thoracolumbar intermediolateral cell column) preganglionic neurons. These fibers arise mainly from neurons of the paraventricular, arcuate, perifornical, and dorsomedial nuclei and the lateral hypothalamus. Pathways between the hypothalamus and autonomic centers are bidirectional; the descending axons are mainly peptidergic (corticotropin-releasing hormone, vasopressin, oxytocin, somatostatin, enkephalin, pro-opiomelanocortin), while the ascending fibers are both peptidergic (enkephalin, neuropeptide Y, neurotensin, dynorphins) and catecholaminergic [119].

The first pharmacological evidence that neuropeptides by central mechanism are able to influence gastric mucosal lesions was provided by the experiments with bombesin. Bombesin, 14-amino acid peptide, inhibited the gastric lesions induced by cold-restraint stress, aspirin, indomethacin by intracisternal (i.c.) and/or intracerebroventricular (i.c.v.) injection [120, 121]. Several mechanism may be involved in the centrally induced mucosal protective effect of bombesin, such as the antisecretory effect [122], inhibition of gastric motor activity, and stimulation of protective processes (gastric mucus, bicarbonate secretion, gastric blood flow) [123].

In addition, neurotensin, β -endorphin, substance P, and somatostatin proved to be also protective given i.c. against cold restraint ulcer model. [124].

Cold restraint stress ulcer model is an acid dependent ulcer model [125]. As a next step, an acid independent model, the ethanol-induced ulcer model has been used to examine the gastroprotective effect of centrally injected neuropeptides, where inhibition of acid secretion is not likely to contribute to the gastric mucosal protective action. Several neuropeptides were injected either into dorsal motor nucleus of vagus (DMNV), i.c. or i.c.v. First, the effect of thyrotropin-releasing hormone (TRH) or its stable analogue RX-77368, injected in low (0-5-1.5 ng), non-secretory dose into the cisterna magna or DMNV was shown to protect the gastric mucosa against ethanol injury [126, 127].

Hereafter, additional neuropeptides proved to be gastroprotective given centrally, among others adrenomedullin [128] peptide YY [129] amylin [130], leptin, cholecystokinin [131], ghrelin [132], opioids, e.g. β -endorphin, deltorphin II, endomorphins [4, 133] nociceptin, nocistatin [134], TLQP-21, a VGF-derived peptide [135], substance P [136] and angiotensin II [137] (see reviews: [4, 127, 138]).

How can the centrally injected neuropeptide induce gastric mucosal protection in the periphery, in gastric mucosa? Convincing evidence suggest the role of vagal nerve in conveying the central stimulus to the periphery. TRH or its stable analogue RX-77368, injected in non-secretory dose into the cisterna magna or DMNV was the first peptide reported to protect the gastric mucosa against ethanol injury through stimulation of vagal cholinergic pathways, since both vagotomy and atropine reversed the gastroprotective effect. As a result of an intensive research additional neuropeptides e.g. amylin, adrenomedullin, neurotensin, opioid peptides, melatonin, ghrelin, leptin, as well as cannabinoids were shown to initiate gastroprotective

effect given centrally (i.c.v., i.c. or DMNV), mostly by a vagal dependent mechanism [139-142] (see reviews: [4,127,138,143, 144]).

The mechanism of vagally mediated gastroprotective effect has been well documented by pharmacological studies, demonstrating that activation of vagal cholinergic pathways stimulates gastric prostaglandin and nitric oxide release and the effector function of capsaicin-sensitive afferent fibers containing CGRP [72, 126, 128,129, 145-147, reviews, 4, 127, 143, 144].

In addition, TRH injected intracisternally at low, gastroprotective dose, stimulates gastric splanchnic afferents as was shown by electrophysiological recording [148] and consequently the local effector function of capsaicin-sensitive splanchnic afferent nerves containing CGRP is activated [128, 149]. Pretreatment with high, neurotoxic dose of capsaicin (125 mg/kg sc.), which induces ablation of primary sensory neurons and the depletion of CGRP-containing fibers, pharmacological blockade of peripheral CGRP₁ receptors by hCGRP 8-37 and NO synthesis by NO-synthase inhibitor N^ω-nitro-L-arginine methyl ester blocked the increased gastric mucosal blood flow induced by i.c. injection of TRH [149-153]. The results indicate that the intact function of sensory fibers containing CGRP and NO is basically important for stimulation of gastric mucosal blood flow and the consequent gastroprotection [152], and the CGRP/NO-mediated gastric hyperemia plays a crucial role to withstand gastric mucosal damage [reviews 61, 154].

Moreover, additional action due to activation of vagal cholinergic pathway might contribute to the mucosal protective effect. Namely, acetylcholine released from efferent nerve terminal of vagal nerve in the periphery, activates $\alpha 7$ subunit-containing nicotinic receptors of macrophages and other immune cells which results in inhibition of the release of proinflammatory cytokines, consequently suppression of inflammation [155, 156].

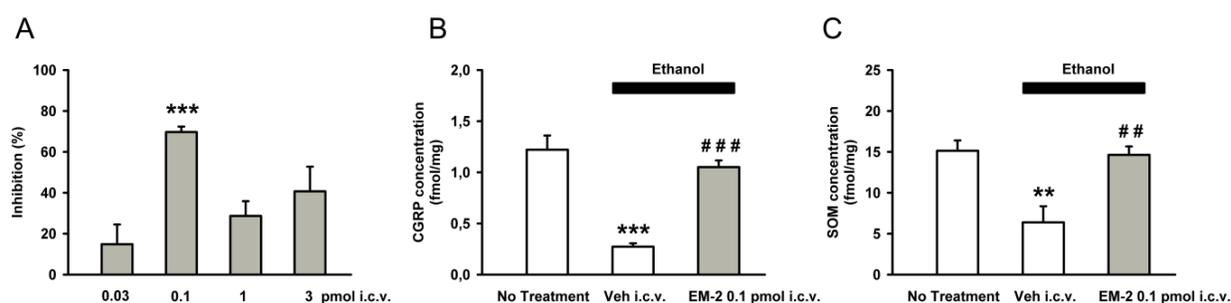
As the above data suggest DVC has a basic role in the regulation of gastrointestinal functions. We wondered if activation of different receptor populations identified in DVC. e.g. μ -opioid receptors (in NTS and DMNV) [157], *cannabinoid receptors* (CB₁-receptors in NTS and DMNV and CB₂-receptors in NTS and brain stem neurons) [158, 159] NK₁-receptors in NTS and DMNV) [160, 161], angiotensin AT₁ receptors in in the NTS, area postrema and DMNV [162, 163] can influence gastric mucosal homeostasis. Our experimental results demonstrated that opioid peptides [4, 133], anandamide, 2-arachidonoyl glycerol (2-AG) endocannabinoids, ligands of cannabinoid CB₁ and CB₂ receptors [137, 142], as well as (SP) [136] given i.c.v. induced gastric mucosal protective effect against ethanol-induced lesions.

We wondered, if in the periphery, sensory neuropeptides, such as CGRP or somatostatin may be involved in the centrally-induced mucosal protective effect of endomorphin-2, endocannabinoids, such as anandamide, 2-AG and SP.

Conclusions on the involvement of CGRP in gastric mucosal protection have been established on pharmacological analysis by using a competitive peptide antagonist of CGRP₁ receptor, CGRP 8-37 or functional ablation of neuropeptides by neurotoxic dose of capsaicin [149, 152]. However, the gastric mucosal level of CGRP or somatostatin has not been determined following central administration of gastroprotective agents.

Therefore we aimed to determine how the gastric mucosal level of CGRP and somatostatin is influenced by the mucosal damaging agent ethanol, and by endomorphin-2, endocannabinoids and SP.

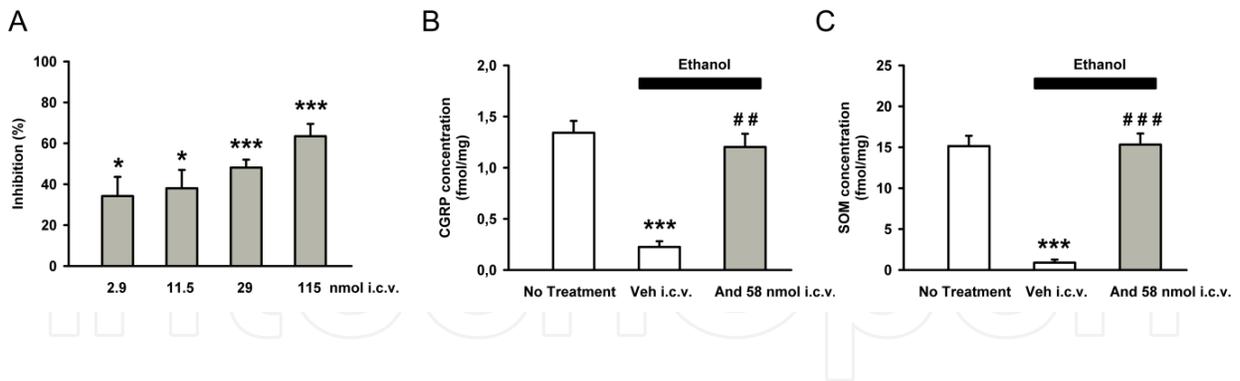
As Fig 1. shows that endomorphin-2 inhibited the ethanol-induced mucosal lesions in the rat, the maximal effect was induced by 0.1 pmol. The mucosal levels of CGRP and somatostatin were decreased in a significant manner following oral administration of ethanol (after 60 min), while i.c.v. injection of endomorphin-2 reversed the decreased levels of both CGRP and somatostatin.



Adapted from Gyires et al. [4].

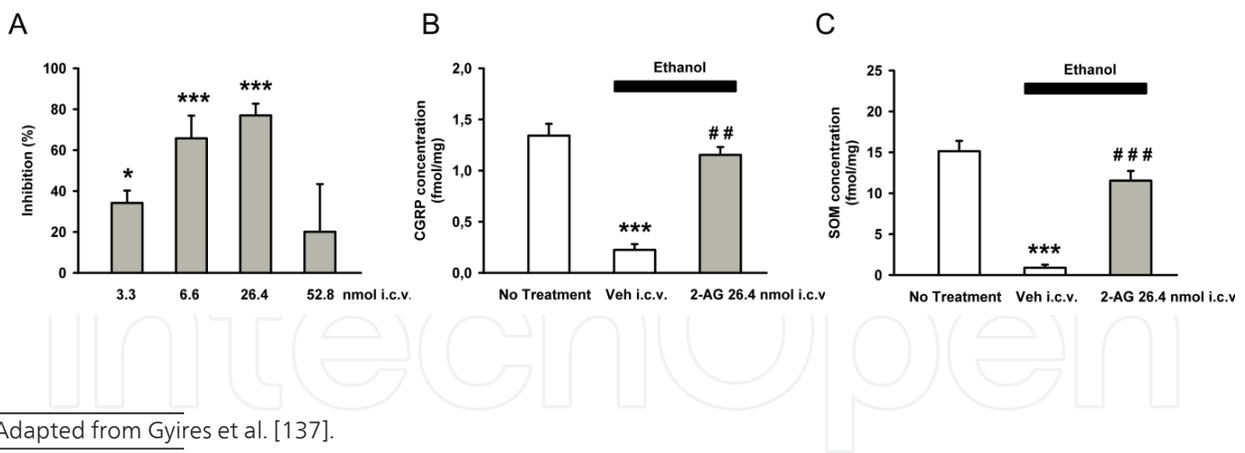
Figure 1. Panel A: The inhibitory effect of endomorphin-2 (EM-2) on the formation of ethanol-induced mucosal lesions in male Wistar rats. Acidified ethanol (2 ml concentrated HCl+98 ml absolute ethanol) was injected per os after 24 h food deprivation in a volume of 0.5 ml/rat. EM-2 was given intracerebroventricularly (i.c.v.) 10 minutes before the ethanol challenge in a volume of 10 μ l in conscious rats. Mucosal lesions were evaluated macroscopically one hour after the administration of ethanol. Each column represents mean \pm S.E.M., the number of rats was 5-10/group. *** p <0.001 compared to the respective control group (vehicle-treated rats) (One-way ANOVA, Newman-Keuls post hoc test). Panels B and C: The effect of EM-2 on the mucosal levels of calcitonin gene-related peptide (CGRP) and somatostatin. CGRP and somatostatin concentrations were determined from gastric mucosal tissue extract samples by means of radioimmunoassay (RIA). Peptide concentrations were calculated as the measured amount of peptide per wet tissue weight, expressed as fmol/mg. Detection limits of the assays were 2 fmol/ml (somatostatin) and 0.2 fmol/ml (CGRP). Each column represents mean \pm S.E.M., the number of rats was 5/group. ** p <0.01, *** p <0.001 compared to the absolute control group (no treatment), ## p <0.01, ### p <0.001 compared to vehicle (saline) i.c.v.+ethanol p.os-treated group (One-way ANOVA, Newman-Keuls post hoc test).

Similarly, anandamide and 2-AG inhibited the mucosal lesions in a dose dependent manner (though the protective effect of 2-AG was diminished in higher, 52.8 nmol dose), and both anandamide and 2-AG increased the ethanol-induced dramatic reduction of the mucosal CGRP and somatostatin levels in the gastroprotective doses (58, and 26 nmol, respectively) (Fig 2. 3.).



Adapted from Gyires et al. [137].

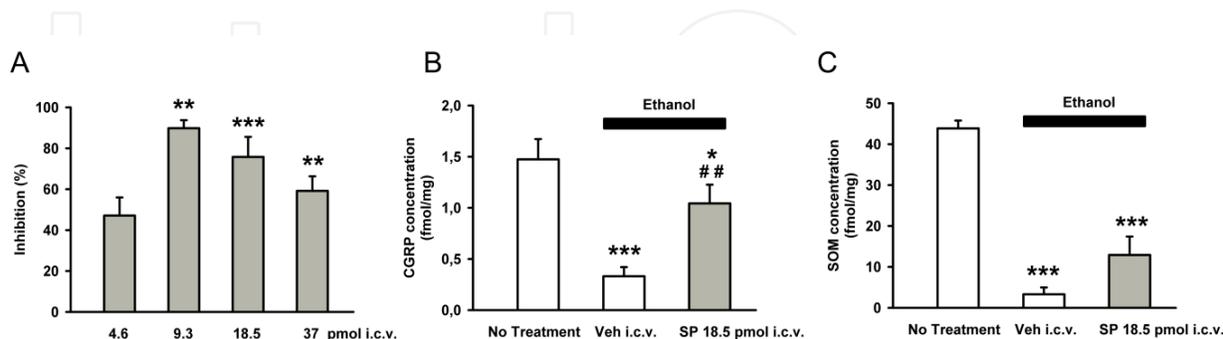
Figure 2. Panel A: The inhibitory effect of anandamide (And) on the formation of ethanol-induced mucosal lesions in male Wistar rats. Methods: see in the Legends of Fig. 1. Each column represents mean ± S.E.M., the number of rats was 5/group. * $p < 0.05$, *** $p < 0.001$ compared to the respective control group (vehicle-treated rats) (One-way ANOVA, Newman-Keuls post hoc test). Panels B and C: The effect of anandamide on the mucosal levels of calcitonin gene-related peptide (CGRP) and somatostatin. Methods: see in the Legends of Fig. 1. Each column represents mean ± S.E.M., the number of rats was 5/group. *** $p < 0.001$ compared to the absolute control group (no treatment), ## $p < 0.01$, ### $p < 0.001$ compared to vehicle (ethanol diluted with saline) i.c.v.+ethanol p.os-treated group (One-way ANOVA, Newman-Keuls post hoc test).



Adapted from Gyires et al. [137].

Figure 3. Panel A: The inhibitory effect of 2-arachidonoylglycerol (2-AG) on the formation of ethanol-induced mucosal lesions in male Wistar rats. Methods: see in the Legends of Fig. 1. Each column represents mean ± S.E.M., the number of rats was 5/group. * $p < 0.05$, *** $p < 0.001$ compared to the respective control group (vehicle-treated rats) (One-way ANOVA, Newman-Keuls post hoc test). Panels B and C: The effect of 2-AG on the mucosal levels of calcitonin gene-related peptide (CGRP) and somatostatin. Methods: see in the Legends of Fig. 1. Each column represents mean ± S.E.M., the number of rats was 5/group. *** $p < 0.001$ compared to the absolute control group (no treatment), ## $p < 0.01$, ### $p < 0.001$ compared to vehicle (acetoneitrile diluted with saline) i.c.v.+ethanol p.os-treated group (One-way ANOVA, Newman-Keuls post hoc test).

SP in pmolar dose range exerted mucosal protective action, the dose response curve proved to be bell shaped, similarly to 2-AG. However, though SP in gastroprotective doses also reversed the ethanol-induced reduction of CGRP mucosal level, it failed to counteract the reduced somatostatin level (Fig. 4.)



Adapted from Brancati et al. [136].

Figure 4. Panel A: The inhibitory effect of substance P (SP) on the formation of ethanol-induced mucosal lesions in male Wistar rats. Methods: see in the Legends of Fig. 1.. Each column represents mean \pm S.E.M., the number of rats was 5/group. ** $p < 0.01$, *** $p < 0.001$ compared to the respective control group (vehicle-treated rats) (One-way ANOVA, Newman-Keuls post hoc test). Panels B and C: The effect of SP on the mucosal levels of calcitonin gene-related peptide (CGRP) and somatostatin. Methods: see in the Legends of Fig. 1. Each column represents mean \pm S.E.M., the number of rats was 5/group. * $p < 0.05$, *** $p < 0.001$ compared to the absolute control group (no treatment), ## $p < 0.01$ compared to vehicle (saline) i.c.v.+ethanol p.os-treated group (One-way ANOVA, Newman-Keuls post hoc test).

Our experimental results provided direct evidence on the role of sensory neuropeptides in centrally initiated gastroprotective effect. It was shown that while ethanol decreased in a significant manner the mucosal CGRP and somatostatin concentration, the centrally injected endomorphin-2, endocannabinoids and SP were able to reverse the highly reduced mucosal levels of sensory neuropeptides supporting the concept on the role of sensory neuropeptides in centrally initiated gastroprotective effect.

4. Conclusion

The apparent redundancy in the mechanisms of gastric mucosal defense might be a potential answer to the question raised above: how can gastric mucosa resist to wide variety of endogenous and exogenous mucosal challenges. The redundancy can be observed in the wide scale of mediators involved in mucosal defense, in generation of endogenous substance(s) to noxious stimulus that may attenuate the mucosal injury and stimulate mucosal repair (for instance, peptide gene expression in gastrointestinal mucosal ulceration [164]), and can induce further protective mechanisms or formation of additional protective mediators.

In the early phase of mucosal injury when the gastric epithelium is exposed to acid or any irritant, one of the most rapid and important responses is the increase in mucosal blood flow to remove, dilute and buffer back-diffusing irritant or acid. In this process primary sensory afferent neurons activated by the back-diffusing acid play a crucial role, since sensory nerve endings can sense acid via acid sensing channel [60-68].

In addition, primary sensory afferent neurons are involved also in the actions of gastroprotective agents. Intensive studies revealed that gastric mucosal defense can be initiated also by central mechanism. Dorsal vagal complex is likely to have prominent role in centrally induced gastroprotective effect. Several neuropeptides, and their receptors e.g. μ -opioid receptors [157-159] NK_1 -receptors [160, 161] angiotensin AT_1 receptors [162, 163] *TRH* receptors [126, 127, 165], neuropeptide Y receptors [166, 167] have been identified in this area, and activation of these receptors result in mucosal protection. Accumulating data suggest that in the periphery the final step in the chain of events of centrally initiated mucosal protective processes is the release of sensory neuropeptides, such as CGRP and partly somatostatin, from the capsaicin sensitive primary afferent neurons, as well as NO and prostaglandins. The process is likely to be vagal dependent, and biochemical and pharmacological studies have shown that vagal cholinergic pathway stimulates gastric prostaglandin and nitric oxide release. In addition, vagal muscarinic mediated release of prostaglandins, histamine and serotonin [147, 150] evoke sensory C-fiber excitation and a subsequent release of neuropeptides from sensory nerve endings.

However, sympathetic nervous system may also be involved in the centrally induced gastroprotective effect, since e.g. the gastroprotective effect of opioid peptides markedly decreased following i.c.v. administration of the catecholaminergic neurotoxin, 6-hydroxydopamine, that reduced the noradrenaline concentration in a significant manner in the NTS[138].

Tache [165] raised that that the redundancy of brain peptides may be linked with different pathophysiological conditions whereby they are recruited into the brain under stress, feeding or damage of the gastric mucosa. Further studies focusing to clarify the role of neuropeptide-interactions in gastroprotection, neuronal projections between brainstem autonomic centers and upper brain areas, additional pathway(s) that may convey central stimulus to the periphery, identification of additional peripheral mechanisms, signaling of peripheral insults of gastric mucosa to the CNS, are some of the opened questions that should be answered and consequently, the better understanding of the central regulation of gastric mucosal homeostasis may result in the development of new strategy and new concept in prevention/treatment of gastric mucosal injuries.

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