Role of Angiotensin-(1–7) in Gastroprotection against Stress-Induced Ulcerogenesis. The Involvement of Mas Receptor, Nitric Oxide, Prostaglandins, and Sensory Neuropeptides

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ABSTRACT

Angiotensin-(1–7) [Ang-(1–7)] is a major vasoactive metabolite of renin-angiotensin system (RAS). The WRS lesions were dose-dependently reduced by pretreatment with Ang-(1–7), which also caused an increase in gastric blood flow (GBF) and NO levels. COX-1 and COX-2 inhibitors (NWS, [5-imino(nitroamino)methyl]-l-ornithine) reversed the reduction in lesion number and the rise in GBF evoked by Ang-(1–7). Ang II augmented the WRS lesions, decreased GBF and increased the plasma IL-1β and TNF-α levels. Capsaicin denervation and COX-2 inhibitors decreased the reduction in Ang-(1–7)-induced gastric lesions and increase in GBF; these effects were restored by supplementation with calcitonin gene-related peptide (CGRP). The mRNA was upregulated while iNOS, IL-1β, and TNF-α mRNAs were downregulated in Ang-(1–7)-pretreated rats. We conclude that Ang-(1–7), in contrast to Ang II, which worsens WRS ulcerogenesis, affords potent gastroprotection against WRS ulcerogenesis via an increase in GBF mediated by NO, endogenous prostaglandins, sensory neuropeptides, and anti-inflammatory action involving the inhibition of proinflammatory markers iNOS, IL-1β, and TNF-α.

INTRODUCTION

The renin-angiotensin system (RAS) is a classic endocrine system involved in physiologic regulation of blood pressure and water and mineral balance (Paul et al., 2006). The components of RAS appear to be functionally active in numerous organs including kidneys, heart, liver, reproductive organs, and skin. Angiotensin I (Ang I) and Ang II play an important role in control of gastrointestinal (GI)-functions such as trophic and electrolyte homeostasis, maintenance of normal blood flow, mucosal absorption of glucose, gastrointestinal motility, mucosal secretion, gastric inflammation, and carcinogenesis (Fandriks 2011; Garg et al., 2012). Recently, the essential Ang I and Ang II metabolites have been identified throughout the GI tract, including stomach, colon, pancreatic islets, and liver (Carl-McGrath et al., 2009; Olszanecki et al., 2009; Hasegawa et al., 2009).

Ang II is the central product of RAS and potent constrictor of vascular smooth muscles (Heinemann et al., 1999). Ang II acts via angiotensin receptor type 1 (AT1) and contributes to vasoconstriction, inflammation, vascular and cardiac hypertrophy, and extracellular tissue remodeling by inhibition of cell growth and stimulation of apoptosis (Lemarie et al., 2009). Stimulation of the AT1 receptors activates membrane cell growth and stimulation of apoptosis (Lemarie et al., 2009).

Ang-(1–7), a major vasoactive metabolite of renin-angiotensin system (RAS), acts via angiotensin receptor type 1 (AT1) and contributes to vasoconstriction, inflammation, vascular and cardiac hypertrophy, and extracellular tissue remodeling by inhibition of cell growth and stimulation of apoptosis (Lemarie et al., 2009; Olszanecki et al., 2009; Hasegawa et al., 2009).
superoxide and hydrogen peroxide (H₂O₂), and inactivates NO pathway (Mehta and Griendling, 2007). Ang II-activating phospholipase C (PLC) and protein kinase C (PKC) or phospholipase A₂ enhanced synthesis of vasoconstrictive leukotrienes and smooth muscle cell contraction (Mehta and Griendling, 2007; Lemarie et al., 2009). Increased reactive oxygen species (ROS) and decreased blood flow play fundamental roles in the pathogenesis of GI mucosal injury (Bregenzio et al., 2003; Nakagiri et al., 2010).

Exposure to stress is commonly recognized as a risk factor of microbleeding and gastric mucosal injury. Reaction to stress is mediated via two distinct but unrelated systems: the hypothalamic-pituitary-adrenocortical (HPA) system and the sympathoadrenal system (Goldstein and McEwen, 2002; Saavedra et al., 2006). Ang II receptor subtypes AT₁ and AT₂ were detected in the human esophageal, gastric, small intestinal, and colonic mucosa (Hirasawa et al., 2002; Casselbrant et al., 2009; Hallersund et al., 2009). The antagonists of Ang II AT₁ receptors attenuated gastric injury induced by ischemia-reperfusion, cold stress, and indomethacin-induced damage in rodents due to an inhibition of sympathetic nervous system activity (Tschöpe et al., 1997) and downregulation of proinflammatory activity of Ang II (Pavel et al., 2004; Morsy et al., 2009; Gemici et al., 2010; Saavedra et al., 2010; Santos et al., 2011).

Angiotensin-(1–7) (Ang-(1–7)) is a heptapeptide generated from angiotensin I (Ang I) by angiotensin-converting enzyme (ACE) homolog AT₂ receptor neutral endopeptidase (NEP), also known as nephrilysin, since the discovery of Ang-(1–7) in 1976, the presence of this heptapeptide has been detected in brain, blood vessels, heart, kidney, liver, and stomach (Santos et al., 2005; Xu et al., 2011). Ang-(1–7) acting via its own G protein-coupled receptor, called Mas (Santos et al., 2009; Stegbauer et al., 2009) exhibits the vasodilatory, antihypertensive, cardio-protective, and antifibrotic effects (Chen et al., 2005). Ang-(1–7) may even precede Ang I conversion to Ang II (Cimanek et al., 2009). Mas receptor knockdown mice present higher expression of eNOS, suggesting a link between Ang-(1–7) and Mas receptor (Xu et al., 2008). The vasoconstrictive action of Ang II in hypertension is limited by vasoactive Ang-(1–7) and bradykinin (Oliveira et al., 2004; Basso et al., 2006; Sampaio et al., 2007). Ang-(1–7) exhibited a protective effect against reflux esophagitis (Papadopoulou et al., 2012). Whether Ang-(1–7) protects the gastric mucosa against stress lesions due to an increase in NO and the activity of prostaglandin (PG)/COX-1 and COX-2 pathways and sensory nerves has not been extensively studied.

We compared the effect of exogenous Ang-(1–7) and Ang II on stress-induced gastric lesions and accompanying changes in the gastric blood flow (GBF). The involvement of endogenous PG and NO as well as the activity of afferent sensory nerves in the mechanism of gastroprotection induced by Ang-(1–7) was investigated by testing the effect of exogenous Ang-(1–7) against stress ulcerogenesis in the presence of NO-synthase inhibitor L-NNA, nonselective and selective COX-1 and COX-2 inhibitors, as well as in rats with capsaicin denervation. We also assessed the effect of Ang-(1–7) on the expression of mRNA for constitutively expressed nitric-oxide synthase (eNOS), inducible nitric-oxide synthase (iNOS), proinflammatory cytokines interleukin (IL)-1β and tumor necrosis factor (TNF)-α, and plasma levels of these cytokines during stress ulcerogenesis.

**Materials and Methods**

**Animals.** Male Wistar rats total 254 with weight averaging about 250 g were used in this study. Rats were fasted for 24 hours with free access to drinking water before exposure to WRS. The study was approved by the Institutional Animal Care and Use Committee of Jagiellonian University Medical College in Cracow and was performed in accordance with the guidelines of the Helsinki Declaration regarding handling of experimental animals.

**Stress-Induced Gastric Lesions, Chemicals, and Drugs.** To induce gastric lesions, rats were immobilized in individual Polman cages and immersed in the cold water (23°C) for 3.5 hours or until the rat body level as reported by our previous studies (Brzozowski et al., 2000; Konturek et al., 2001). Three major experimental groups of rats (A-G) were selected. Thirty minutes before exposure to water immersion and restraint stress (WRS), rats in series A–C received pretreatment with one of the following: (A) exogenous Ang-(1–7) (0.01–0.05 μg/kg i.p.), the nonpeptide Ang-(1–7) Mas receptor antagonist (Bayohr et al., 1999; Santos et al., 2009; Santos et al., 2011) or perindopril (8 mg/kg i.p.), the ACE inhibitor (Agiel et al., 2012). The angiotensin Mas receptor agonistic and antagonist activities were determined in a separate group of rats (series D) treated with A-779 (5 mg/kg i.p.), the selective Ang-(1–7) Mas receptor antagonist (Bayohr et al., 1999; Santos et al., 2009; Santos et al., 2011) or with combination of Ang-(1–7) Mas and perindopril (8 mg/kg i.p.), nonpeptide Ang-(1–7) receptor agonist (Pinheiro et al., 2004; Santos and Fereira, 2006), respectively.

In separate experiments, the effects of treatment with Ang-(1–7) or perindopril, combined with i-NNA (20 mg/kg i.p.), the selective inhibitor of NO-synthase activity, on WRS lesions and alterations in the GBF were determined.

**Reagents and Drugs.** All tested drugs and compounds were of analytical grade and were purchased from Sigma-Aldrich Laborchemikalien (Schelldorf, Germany) except of SC-560 and rofecoxib purchased from Cayman.
Gastroprotection by Angiotensin-(1–7) Against Stress Damage

Moloney murine leukemia virus reverse transcriptase (MMLV-RT), DNA (cDNA) in a 50-°C reaction volume containing 0.3 μl (2.5 IU) Taq polymerase, 1.5 mM (each) dNTP (Pharmacia, Germany), 1.5 mM KCl, 5 ml Taq polymerase chain reaction buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3), and primers used at final concentration of 0.5 μM. The mixture was overlaid with 25 μl of mineral oil to prevent evaporation. The polymerase chain reaction mixture was amplified in a DNA thermal cycler (Perkin-Elmer/Cetus, Norwalk, CT) using a temperature program as described in detail in our previous studies (Brzozowski et al., 2004, 2006; Kwiecien et al., 2007). The GBF measured by means of H2-gas clearance technique as reported (Konturek et al., 2009). The signals for cNOS, IL-1β, and TNF-α were standardized against the β-actin signal for each sample, and results were expressed as cNOS, iNOS, and TNF-α mRNA/β-actin mRNA ratios.

Statistical Analysis. All results of the experiment were expressed as mean ± S.E.M. and the statistical analysis was performed with two-way analysis of variance (ANOVA) test and Tukey post hoc test where appropriate. Differences between estimates of effects were considered significant at P < 0.05. All results in the treated animals were compared with the appropriate control group, which had been established for each set of experiments. Dependent variables were expressed both in percentage of control for GBF and in absolute values for lesion number. The control rats did not differ from experimental animals in terms of relevant characteristics, such as source of purchase, gender, age, weight, diet, and housing conditions. There was no random pairing of animals, the paired statistical tests were not used.

Results

Mean Lesion Number and GBF in Rats Pretreated with Ang II or Ang(1–7). Exposure of vehicle-pretreated control rats to 3.5 hours of WRS caused gastric mucosal lesions (hemorrhagic erosions) accompanied by a significant fall in GBF (Fig. 1). The pretreatment with Ang II applied in a dose of 5 μg/kg failed to significantly affect the mean lesion number and GBF compared with vehicle-control.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence</th>
<th>Annealing Temperature</th>
<th>Size of PCR Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-NOS</td>
<td>Forward: 5'-TAC GGA GCA GCA AAT CCA C-3', Reverse: 5'-CAG CTT GCA GTC CTT TCA TC-3'</td>
<td>63,5</td>
<td>540</td>
</tr>
<tr>
<td>IL1-β</td>
<td>Forward: 5'-GCT ATT GTC TTT CCC GT-3', Reverse: 5'-GAC CAT TCG TCT TCC GTA G-3'</td>
<td>62</td>
<td>543</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Forward: 5'-TAC TGA ACT TCG GGG TGA TTT GTC C-3', Reverse: 5'-CAG CCT TGG CTT AAG AGA ACC-3'</td>
<td>56</td>
<td>295</td>
</tr>
<tr>
<td>β-Actin</td>
<td>Forward: 5'-TTG TAA CCA ACT GGG ACA ATA TGG-3', Reverse: 5'-GAT CTT GAT CTT CAT GGT GCT AGG-3'</td>
<td>54</td>
<td>764</td>
</tr>
<tr>
<td>iNOS</td>
<td>Forward: 5'-CCA CAA TAG TAC AAT ACT AC-3', Reverse: 5'-ACG AGG TGT TCA CGG TGC TGC-3'</td>
<td>60</td>
<td>397</td>
</tr>
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Dose of Ang-(1–7) increased in GBF and luminal NO concentration (Fig. 2). The lesions, while producing a significant and a dose-dependent
m
administration i.p. in graded doses ranging from 6.25 to 40 \( \mu g/kg \). Results are mean ± S.E.M. from seven animals per each experimental group. The Ang II-pretreated groups were compared with vehicle-pretreated animals as indicated under Materials and Methods. Asterisk indicates a significant change (\( P < 0.05 \)) compared with the respective values in Veh-controls.

The administration of Ang II in higher doses ranging from 6.25 to 40 \( \mu g/kg \) dose-dependently increased the lesion number and produced a significant, dose-dependent decrease in GBF (Fig. 1). The pretreatment with Ang-(1–7) administered i.p. in graded doses ranging from 6.25 to 50 \( \mu g/kg \), dose-dependently attenuated WRS-induced gastric lesions, while producing a significant and a dose-dependent increase in GBF and luminal NO concentration (Fig. 2). The dose of Ang-(1–7) inhibiting WRS lesions by 50\% (ID\(_{50}\)) was 27 \( \mu g/kg \). Since the dose of 50 \( \mu g/kg \) afforded the maximal protective response (\( P < 0.05 \)), this dose of Ang-(1–7) was used in all our determinations. The absolute values for GBF expressed in ml/min per 100 g are presented in Table 2. Exposure to WRS in rats pretreated with vehicle-control significantly decreased the GBF (\( P < 0.05 \)) compared with the values in the intact gastric mucosa. This decrease in GBF under WRS conditions was significantly worsened by the pretreatment with Ang II. In contrast, the pretreatment with Ang-(1–7) resulted in a significant increase in the GBF (\( P < 0.05 \)) compared with the vehicle-pretreated with vehicle. The Ang-(1–7)-induced protective effect accompanying rise in the GBF and luminal NO content was observed at the 50 \( \mu g/kg \) dose of this peptide; but completely reversed by the pretreatment with A-779 (50 \( \mu g/kg \) i.p.) combined with intraperitoneal treatment with Ang-(1–7) (Fig. 2; Table 2).

Effect of AVE 0991, the Agonist of Ang-(1–7) Mas Receptor, on WRS-Induced Gastric Lesions and Alterations in the GBF. As shown in Fig. 3, the pretreatment with AVE 0991 (50 \( \mu g/kg \) i.p.) significantly reduced the mean lesion number (\( P < 0.05 \)) and a significantly decrease in the GBF (\( P < 0.05 \)) compared with the respective values in vehicle-control pretreated rats. The decrease in lesion number and an increase in the GBF induced by AVE 0991 were completely reversed by the combination of A-779 and AVE 0991 (\( P < 0.05 \)).

Effect of Suppression of NO-Synthase on Ang-(1–7)- and Perindopril-Induced Gastroprotection and Alterations in GBF in Rats Exposed to WRS. Figure 4 shows that pretreatment with Ang-(1–7) (50 \( \mu g/kg \) i.p.) significantly reduced the WRS-induced gastric lesions and increased GBF, with the effect similar to the respective values presented in Fig. 2. The pretreatment with perindopril (5 mg/kg i.p.) also significantly decreased the number of WRS-induced gastric lesions (\( P < 0.05 \)) and significantly increased GBF compared to vehicle-control. Administration of L-NNA (20 mg/kg i.p.), which is known to significantly affect the lesion number and GBF compared to vehicle-treated control, reversed the attenuation in lesion number and the rise in GBF evoked by Ang-(1–7) or perindopril (Fig. 4).

Effect of COX-1/PG and COX-2/PG Suppression on Ang-(1–7)-Induced Gastric Protection against WRS-Induced Gastric Damage and Alteration in GBF. As shown in Fig. 5, the pretreatment with Ang-(1–7) (50 \( \mu g/kg \) i.p.) caused a similar decrease in the mean number of WRS-induced lesions (\( P < 0.05 \)) compared to vehicle-control. The inhibition of COX-2/PG suppressed the protective effect exerted by Ang-(1–7) (Fig. 5; Table 2). The inhibition of COX-1/PG, however, had a significant protective effect on the lesion number (\( P < 0.05 \)) and the increase in GBF (\( P < 0.05 \)) compared to the respective values after Ang-(1–7) alone.

**Table 2**

| Type of Test | GBF
<table>
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<tr>
<td><strong>ml/min per 100 g</strong></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>46 ± 2.8</td>
</tr>
<tr>
<td>Veh + WRS</td>
<td>27 ± 2.2*</td>
</tr>
<tr>
<td>Ang II + WRS</td>
<td>21 ± 1.6**</td>
</tr>
<tr>
<td>Ang-(1–7) + WRS</td>
<td>35 ± 2.7**</td>
</tr>
<tr>
<td>A-779 + Ang-(1–7) + WRS</td>
<td>26 ± 2.2*</td>
</tr>
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fall in GBF (Fig. 5). The addition of PGE2 (5 mg/kg i.p.), rofecoxib (10 mg/kg i.g.), and SC-560 (5 mg/kg i.g.) significantly attenuated by pretreatment with indomethacin (INDO), SC-560 (SC), and rofecoxib (ROFE) with and without supplementation with prostaglandin E2 (PGE2). Results are mean ± S.E.M. from seven animals per each experimental group. The values in AVE 0991 group were compared with vehicle-controls and with those in AVE 0991 given in combination with A-779. Asterisk indicates a significant change (P < 0.05) compared with the respective values in vehicle-control group. Cross indicates a significant change (P < 0.05) compared with respective values in vehicle high-control group. The reduction of lesion number by Ang-(1–7) pretreatment with COX-1 and COX-2 inhibitors alone significantly increased the mean lesion number and produced a significant difference compared with vehicle-treated animals exposed to WRS (data not shown). The reduction of lesion number by Ang-(1–7) (50 μg/kg i.p.) was substantially attenuated by pretreatment with indomethacin (5 mg/kg i.p.), rofecoxib (10 mg/kg i.g.), and SC-560 (5 mg/kg i.g.) (P < 0.05), and these effects were accompanied by a significant fall in GBF (Fig. 5). The addition of PGE2 (5 μg/kg i.g.) to Ang-(1–7) restored the gastroprotective effect of this peptide in the presence of COX-1 and COX-2 inhibitors (P < 0.05), and these effects were accompanied by an increase in GBF similar to that recorded in Ang-(1–7)-treated animals without concomitant treatment with COX inhibitors. Double asterisk indicates a significant change (P < 0.05) compared to the values obtained in group treated with INDO, SC, and ROFE in the presence of Ang-(1–7) but without combination with PGE2.

Fig. 3. Mean lesion number and the alterations in GBF in gastric mucosa pretreated with vehicle (Veh; control) or AVE 0991 (50 μg/kg i.p.), the Ang-(1–7) receptor agonist, without or with A-779 (the antagonist of Mas receptors; 50 μg/kg i.p.) and exposed to WRS. Results are mean ± S.E.M. from six animals per each experimental group. The values in AVE 0991 group were compared with vehicle-controls and with those in AVE 0991 given in combination with A-779. Asterisk indicates a significant change (P < 0.05) compared with the respective values in vehicle-control group. Cross indicates a significant change (P < 0.05) compared to the respective values in vehicle high-control group. The reduction of lesion number by Ang-(1–7) pretreatment with COX-1 and COX-2 inhibitors alone significantly increased the mean lesion number and produced a significant difference compared with vehicle-treated animals exposed to WRS (data not shown). The reduction of lesion number by Ang-(1–7) (50 μg/kg i.p.) was substantially attenuated by pretreatment with indomethacin (5 mg/kg i.p.), rofecoxib (10 mg/kg i.g.), and SC-560 (5 mg/kg i.g.) (P < 0.05), and these effects were accompanied by a significant fall in GBF (Fig. 5). The reduction in lesion number and an increase in the GBF caused by Ang-(1–7) in rats with intact sensory nerves were almost completely lost in those with capsaicin denervation. The concurrent administration of CGRP combined with Ang-(1–7) significantly reduced the mean lesion number (P < 0.05) and significantly increased GBF in capsaicin-denervated rats (P < 0.05); however, these values were still significantly different from those attained with Ang-(1–7) in rats with intact sensory nerves (Fig. 6).

Effect of Pretreatment with Ang-(1–7) or Ang II on Plasma Levels of Proinflammatory Cytokines IL-1β and TNF-α in Rats Exposed to WRS. As shown in Fig. 7, the plasma levels of IL-1β and TNF-α were negligible in intact rats not exposed to WRS. In contrast, the plasma TNF-α and
IL-1β levels were significantly increased in vehicle-treated rats exposed to WRS (P < 0.02). A further significant rise in plasma level of IL-1β and TNF-α was observed in the group administered with Ang II (50 μg/kg i.p.) compared with those pretreated with vehicle (Fig. 7). In contrast, the plasma levels of IL-1β and TNF-α significantly decreased in animals treated with Ang-(1-7) (50 μg/kg i.p.) significantly compared to the values obtained in animals with intact sensory nerves treated with Ang-(1-7). Cross indicates a significant change (P < 0.05) compared with the respective values in intact sensory nerves and in those with functional ablation of sensory nerves by capsaicin (capsaicin denervation). In contrast, strong signals for IL-1β mRNA were significantly upregulated in WRS-induced gastritis mucosa. These effects were significantly attenuated in animals pretreated with Ang-(1-7) (Fig. 8, right panel).

Figure 9 (upper panel) demonstrates that the signal for iNOS mRNA was negligible in the intact gastric mucosa, but increased in those pretreated with vehicle (Veh; control). The ratio of iNOS mRNA over β-actin confirmed that mRNA for iNOS was significantly increased in rats exposed to WRS when compared with that in the intact gastric mucosa and this effect was significantly attenuated in those pretreated with Ang-(1-7). The ratio of iNOS mRNA over β-actin confirmed that mRNA for iNOS was significantly increased in rats exposed to WRS when compared with that in the intact gastric mucosa and this effect was significantly attenuated in those pretreated with Ang-(1-7).

Figure 9 (left panel) shows the expression of cNOS mRNA in gastric mucosa of vehicle-pretreated group and those administered with Ang-(1-7) with or without cotreatment with exogenous CGRP and with or without capsaicin denervation as indicated under Materials and Methods. Asterisk indicates a significant change (P < 0.05) compared with the respective values in intact sensory nerves and in those with functional ablation of sensory nerves by capsaicin (capsaicin denervation).
**Discussion**

Our study indicates for the first time that Ang-(1–7), one of the major metabolites of Ang II, contributes to the mechanism of gastroprotection in gastric mucosa induced by stress, which is one of the important risk factors for peptic ulcer, hemorrhagic erosions, and microbleedings in animals and humans (Pavel et al., 2008; Konturek et al., 2011). We have shown that parenteral administration of Ang-(1–7) ameliorated in a dose-dependent manner the severity of WRS-induced gastric lesions and this effect was accompanied by the increase in interarterial and luminal NO content. Blockade of Mas receptor by A-779 inhibited the Ang-(1–7)-induced protection and hyperemia, while AVE 0991, the agonist of Ang-(1–7) receptors, mimicked the gastroprotective and hyperemic actions of Ang-(1–7). Our results provide the evidence that NO-NOS system and PG-COX pathways could be involved in the protective and hyperemic activities of this Ang II metabolite because this protection and an increase in GFB were reversed by the NOS activity inhibitor L-NNA, and by either nonselective or selective COX-1 and COX-2 inhibitors. We have demonstrated that these protective and hyperemic effects of Ang-(1–7), which disappeared in COX-1 and COX-2-treated animals, have been restored by PGE2 coadministered with this peptide in the presence of COX-1 and COX-2 inhibitors. The involvement of NO in gastroprotection and the hyperemic actions of Ang-(1–7) is further supported by the fact that expression of cNOS was upregulated while expression of iNOS, considered as proinflammatory marker, was downregulated in the gastric mucosa of Ang-(1–7)-pretreated rats. This gastroprotective and hyperemic effect of Ang-(1–7) was similar to those exhibited by perindopril, a long lasting ACE inhibitor. The protective and hyperemic effects of Ang-(1–7) were lost in rats with capsaicin denervation consistent with the notion that this peptide may trigger the sensory afferent endings to release vasodilatory and protective CGRP. Indeed, the pretreatment with CGRP enhanced the protective activity of this Ang I metabolite, resulting in gastric hyperemia but also counteracted the capsaicin-induced gastric impairment and the accompanying fall in the gastric GFB observed in...
Ang-(1–7)-treated rats with deactivated sensory nerves. These findings indicate that sensory neuropeptide CGRP can cooperate with PG and NO in the mechanism of Ang-(1–7)-induced gastroprotection and gastric hyperemia against WRS-induced gastric lesions (Fig. 10).

Since stress causes gastric damage of poorly recognized mechanism and etiology, and RAS has been implicated in the pathogenesis of gastric mucosal integrity (Brzozowski et al., 2012) and stress ulcerogenesis (Ender et al., 1993; Kwiecien et al., 2007; Konturek et al., 2011), we determined the effect of vasoactive Ang-(1–7) against stress-induced gastric lesions and compared it with that of Ang II. In clear contrast to Ang-(1–7), the pretreatment with Ang II failed to exert gastroprotection and exacerbated the WRS-induced gastric lesions accompanied by the fall in the GBF. Moreover, Ang-(1–7) markedly decreased the expression and release of proinflammatory cytokines IL-1β and TNF-α (Szalachic et al., 2013) suggesting that the anti-inflammatory properties of Ang-(1–7) contribute to protective activity of this Ang I metabolite in the rat stomach (see Fig. 10).

Previous studies documented that AT1-receptor antagonists help to maintain the proper gastric blood perfusion via the reduction of sympathetic neural activity and attenuation of inflammatory mediators (Ender et al., 2003; Konturek et al., 2011; Garg et al., 2012). Bregonzio et al. (2004) observed the reduction of sympathetic neural activity and by attenuants help to maintain the proper gastric blood perfusion via mediated by COX/PG system responsible for the attenuation of malondialdehyde content and rise in superoxide dismutase activity. The intestinal mucosal COX-2 expression is decreased in abdominal fat of overweight rats (Saavedra et al., 2005, 2006). It is not excluded that the beneficial effect of AT1-receptor antagonists could depend on enhancement of the concentration of angiotensin metabolites Ang-(1–7) and Ang-(7–9) (Neves et al., 2000; Olszanecki et al., 2009), but the hypothesis requires further studies.

Our results show that WRS increased the expression and plasma levels of TNF-α and IL-1β and that a plasma level of these proinflammatory cytokines was increased by Ang II, suggesting that Ang-(1–7) is, in contrast to Ang II, known as a potent vasoconstrictor. Moreover, WRS-induced gastric damage due to its proinflammatory action. This is corroborative with the observations that high levels of circulating Ang-(1–7), especially in adipose tissue (Saavedra et al., 2012), Ang-(1–7) decreased body weight, increased HDL cholesterol, and decreased expression of COX-2 and IL-1β in abdominal fat of overweight rats (Saavedra et al., 2012). Moreover, Clarção et al. (2001) reported a direct binding of Ang-(1–7) to the Ang I receptor, leading to down regulation of these receptors. In keeping with these findings, we observed decreased expression and plasma levels of IL-1β and TNF-α in rats pretreated with Ang-(1–7), suggesting that there is a clear difference between Ang-(1–7) and Ang II with respect to proinflammatory cytokines. Moreover, the endogenous Ang II could contribute to pathogenesis of cold-restraint stress ulcer in obstructive jaundice rats (Mou et al., 1997). Enalapril, an inhibitor of ACE, reduced both the stomach and gastric mucosal Ang II level, decreased gastric blood flow, and increased the extent of mucosal damage (Mou et al., 1998). Furthermore, Ang-(1–7) attenuated ischemia-reperfusion damage is mediated by COX/PG system responsible for the attenuation of malondialdehyde content and rise in superoxide dismutase activity. The intestinal mucosal COX-2 expression is regulated by both AT1 and AT2 receptors (Tani et al., 2008). Ang-(1–7) stimulated PGE2 release from spontaneously hypertensive rat vascular smooth muscle cells (Jaiswal et al., 1993). In our study, the gastroprotection and increase of

**Fig. 10.** Proposed mechanisms and factors that may contribute to the gastroprotective action of vasoactive angiotensin metabolite, Ang-(1–7). RAS metabolite Ang-(1–7) acts via specific Mas receptor and stimulates mucosal protective mechanisms due to an activation of endogenous Mas receptor and stimulates mucosal protective mechanisms due to an activation of NO/NOS and COX/PG systems, sensory neuropeptides such as CGRP released from sensory nerves, and the potent inhibition of proinflammatory cytokines and gastric mucosal inflammation.
Gastroprotection by Angiotensin-(1-7) Against Stress Damage

GBF evoked by Ang-(1-7) were counteracted by pretreatment with COX-1 and COX-2 inhibitors. For many years, PGs have been considered major cytoprotective mediators that play an important role in various aspects of gastroduodenal protection and ulcer healing (Robert, 1979; Tarnawski et al., 1988; Brzozowski et al., 2006; Takeuchi, 2010). Youssif et al. (2012) revealed that PGs are important mediators of the beneficial effects of Ang-(1-7) in cardiac recovery and vascular reactivity in diabetes. Herein, exogenous PGE2 added to Ang-(1-7) in the presence of COX-1 and COX-2 inhibitors restored the gastroprotective and hyperemic activities of this metabolite. Thus, the mechanism through which the Ang-(1-7)/Mas receptor axis induced gastroprotection depends on the activation COXPG system and endogenous PG.

Sensory nerves were implicated in the mechanism of gastroprotection against various gastric damaging factors, including stress and Helicobacter pylori lipopolysaccharide (LPS) (Brzozowski et al., 2004; Kwiecien et al., 2007). The gastroprotective and hyperemic activities of Ang-(1-7) were markedly impaired in rats with capsaicin-induced functional ablation of sensory fibers. This indicates that besides NO and PG affenter sensory fibers and the major sensory neuropeptides CGRP released from rat sensory nerve endings might mark the gastroprotective and hyperemic activities of this metabolite. Thus, the mechanism through which the Ang-(1-7)/Mas receptor axis induced gastroprotection depends on the activation COXPG system and endogenous PG.

References
Concluded experiments: Magierowski, Jasnos, Pawlik, Kwiecien.
Contributed new reagents or analytic tools: Magierowski, Kwiecien, Brzozowski.