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 the renin-angiotensin system (RAS). Ang-(1–7) acts via the Mas receptor, which was documented in kidneys, heart, brain, and gastric tract (GI). We studied the gastrointestinal (GI) tract. We studied the effect of exogenous Ang-(1–7) gavage on gastric blood flow (GBF), gastric mucosal content of NO. COX-1 and COX-2 inhibitors or L-NNA (5-[imidazolyl(4H)]-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 attenuated the reduction of Ang-(1–7)-induced gastric lesions and increase in GBF; these effects were restored by supplementation with calcitonin gene-related peptide (CGRP). The cNOS 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 via an increase in GBF and NO, reduced gastric lesions, and anti-inflammatory action involving the inhibition of proinflammatory markers iNOS, IL-1β, and TNF-α.

INTRODUCTION

The renin-angiotensin system (RAS) is a hormone-gene system involved in physiologic regulation of fluid and electrolyte balance (Paul et al., 2006). Its components of RAS appear to be functionally active in numerous organs including kidneys, heart, brain, and reproductive organs, and skin. Angiotensin I (Ang I) and Angiotensin II (Ang II) play an important role in control of gastrointestinal (GI) functions such as fluid and electrolyte homeostasis, maintenance of gastrointestinal blood flow, and mucosal absorption of glucose, gastrointestinal motility, mucosal secretion, gastric inflammation, and carcinogenesis (Pandriks 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 potentiates constriction 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 NADPH oxidase in vascular smooth muscle cells (VSMCs), enhancing the production of reactive oxygen species such as superoxide anions.
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 (Bregenzo 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-adrenomedullary axis and the attenuation of vasoconstrictor and proinflammatory activity of Ang II (Pavel et al., 2000; Harst et al., 2009; Gemici et al., 2010; Saavedra et al., 2011).

Angiotensin-(1–7) [Ang-(1–7)] is a short-lived peptide generated from angiotensin I by the renin-angiotensin-aldosterone system (RAAS) via the angiotensin-converting enzyme (ACE) homolog ACE2 or neutral endopeptidase (NEP, also known as neprilysin). The discovery of Ang-(1–7) in 1976, the presence of this short peptide has been detected in brain, blood vessels, heart, kidney, liver, and stomach (Santos et al., 2006; Stegbauer et al., 2007). Ang-(1–7) acting via its own G protein-coupled receptor, called Mas (Santos et al., 2006; Stegbauer et al., 2007) exhibit the vasodilatory, antihypertensive, cardioselective, antifibrotic effects, and Ang I is quickly degraded to Ang-(1–7) in the rat stomach, in the formation of Ang-(1–7) in the liver, Ang-(1–7) may even precede Ang I conversion to Ang II (Gazdecki et al., 2009). Mas receptor knockouts decreased NO synthase expression due to dysfunction of endothelial nitric oxide synthase (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., 2001; Liao et al., 2006; Sampaio et al., 2007). Ang-(1–7) exhibits protective action against reflux esophagitis (Rodrigue et al., 2012). Whether Ang-(1–7) protects the gastrointestinal tract against stress lesions due to an increase of NO and the action of prostaglandin (PG)/COX-1 and PG/COX-2 pathways and sensory nerves has been extensively studied in recent years.

We compared the effects of endogenous 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 250 g were used in the 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 conducted in accordance with the statements of the Helsinki Declaration regarding handling and use of experimental animals.

Stress-Induced Gastric Lesions, Chemicals, and Drugs

To induce gastric lesions, rats were immobilized in individual Polman cages and immersed in water at a constant (23°C) for 3.5 hours. The rat xyphoid level as reported by our group previously (Brzozowski et al., 2000, 2006; Satoh et al., 2013). In another experimental group of rats (series D) treated with A-779 (5 mg/kg i.p.), the selective Ang-(1–7) receptor antagonist (Bayoh et al., 1999; Santos et al., 2006; Shiotani et al., 2006) without or with the combination with Ang-(1–7) or perindopril 0.5 mg/kg i.p., the nonpeptide Ang-(1–7) receptor agonist (Pinheiro SV et al., 2004; Santos and Fereira, 2006), respectively.

In series G, the effects of cotreatment with Ang-(1–7) or perindopril, with or without the combination with i-NNA (20 mg/kg i.p.), the selective inhibitor of NO-synthase activity, on WRS lesions and plasma levels of NO were determined. In series F we measured endogenous PG in the gastroprotective effects of perindopril (Ang-(1–7) or vehicle (control) was investigated in rats (F) treated with indomethacin (5 mg/kg i.p.), the nonselective COX-1 and COX-2 inhibitor, or SC-560 (5 mg/kg i.p.), the selective inhibitor of COX-1, and rofecoxib (10 mg/kg i.p.), the selective inhibitor of COX-2 activity as reported in our previous studies (Brzozowski et al., 2000, 2006; Satoh et al., 2013). In another subgroup with COX-1 and COX-2 inhibitors, rats of series F were coadministered with exogenous prostaglandin E₂ (PGE₂; 5 μg/kg i.g.) in the presence of Ang-(1–7).

In series G, the effect of blockade of sensory nerves induced by large dose of capsaicin (total 125 mg/kg s.c.) on the protective and hyperemic activity of Ang-(1–7) was examined. Capsaicin was injected for 3 consecutive days at a respective dose of 25, 50, and 50 mg/kg s.c. approximately 2 weeks before the experiment to induce the functional ablation of sensory nerves as described previously (Konturek et al., 2009; Kwiecien et al., 2012a). In separate subgroup of series G with capsaicin denervation, the involvement of calcitonin gene–related peptide (CGRP), the major rat neuropeptide released from sensitive afferent nerve endings in protective action of exogenously administered Ang-(1–7) against WRS lesions, was determined. In one of the subgroups of series G, the capsaicin-denervated rats received supplementation with exogenous CGRP (10 μg/kg s.c.) combined with Ang-(1–7) and 30 minutes later were exposed to onset of WRS as in other groups described above.

All tested drugs and compounds were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA) except of SC-560 and rofecoxib purchased from Cayman Chemicals.
Measurement of GBF and Determination of Gastric Lesion Number. At the termination of 3.5 hours WRS, rats were anesthetized with pentobarbital (60 mg/kg i.p.), the abdomen was opened, and GBF measured by means of H₂gase clearance technique as reported before (Brzozowski et al., 2004, 2006; Kwiecien et al., 2007). The GBF was measured in the fundic part of the gastric mucosa not involving mucosal lesions. Average values of three measurements were determined and expressed as a percentage of change of the value determined in intact rat stomach. Gastric lesions number was determined on photographed stomachs with computerized planimetry (Morphomat, Carl Zeiss, Berlin, Germany) (Kwiecien et al., 2012a) by a blinded investigation.

Determination of Luminal NO Content and Plasma Level of IL-1β and TNF-α. The luminal concentration of NO was quantified indirectly as nitrate (NO₃⁻) and nitrite (NO₂⁻) levels in the gastric contents using the nitrate/nitrite kit purchased from Cayman Chemical as described in detail in our previous studies (Brzozowski et al., 2008; Pawlik et al., 2011; Kwiecien et al., 2012b).

The blood samples (~3 ml) were taken from the vena cava for the measurement of plasma proinflammatory cytokines IL-1β and TNF-α as described previously (Kwiecien et al., 2012b). In brief, the plasma sample (50 μl) was incubated with biotinylated antibodies specific for TNF-α and IL-1β was determined by a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA; BioSource International Inc., Camarillo, CA) according to the manufacturer’s instructions. The luminal NO content and TNF-α and IL-1β were standardized against the β-Actin mRNA ratio. The expression mRNA of cNOS, iNOS, IL-1β, and TNF-α in the rat gastric mucosa determined by reverse transcription-polymerase chain reaction (RT-PCR) with specific primers. Mucosal specimens were scraped using a glass slide and immediately snap-frozen in liquid nitrogen and stored at −80°C until analysis. Total RNA was extracted from mucosal sample by a guanidium isothiocyanate/phenol chloroform method using a kit from Stratagene (Heidelberg, IL), 2 ml of a 100 mM mixture of deoxyadenosine triphosphate (dATP), deoxythymidine triphosphate (dGTP), deoxyguanosine triphosphate (dTTP), and deoxycytidine triphosphate (dCTP), 5 ml of 10× RT buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 5 mM MgCl₂). The resultant cDNA (2 μl) was amplified in a 50-μl reaction volume containing 0.3 μl (2.5 IU) Taq polymerase, 2 μM (each) dNTP (Pharmacia, Germany), 1.5 mM MgCl₂, 5 ml of Taq polymerase reaction buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3), and primers used at final concentrations of 0.5 μM. Each reaction mixture was overlaid with 25 μl of mineral oil to prevent evaporation. The polymerase chain reaction mixture was amplified on a DNA thermal cycler (Perkin-Elmer/Cetus, Norwalk, CT) in the area dedicated for performing PCR reaction. The specificity of the selected reaction products was confirmed by using DNA 100-bp ladder (Gibco) as a standard size marker. The intensity band was quantified using densitometry (LKB Ultrascan, Pharmacia, Uppsala, Sweden) as described in detail in our previous studies (Brzozowski et al., 2008; Kwiecien et al., 2009). The signals for cNOS, iNOS, IL-1β, and TNF-α were standardized against the β-Actin signal for each sample, and results were expressed as cNOS/β-Actin, iNOS/β-Actin, and TNF-α mRNA/β-Actin.

Statistical Analysis. 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 in mean 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 as percentage of control for GBF and in absolute values for lesion number. The control rats did not differ from experimental groups in terms of relevant characteristics, such as source of purchase, gender, age, weight, diet, and housing conditions. There was no individual pairing of animals, the paired statistical analysis was 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>Forward</th>
<th>Reverse</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', 5'-CAG GCT GCA GTC CTT TGA TC-3'</td>
<td>63.5</td>
<td>540</td>
<td></td>
</tr>
<tr>
<td>IL1-β</td>
<td>Forward: 5'- GCT ACC TAT GTG TTT CCC GT-3', 5'-GAC CAT TCG TGG TTC CTA GG-3'</td>
<td>62</td>
<td>543</td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>Forward: 5'- TAC TGA ACT TCG GGG TGA TGG TGC C-3', 5'-CAG CTT CCG TGG AAG AGA ACC-3'</td>
<td>56</td>
<td>295</td>
<td></td>
</tr>
<tr>
<td>β-Actin</td>
<td>Forward: 5'- TGG TAA CCA ACT GGG ACG ATA TGG-3', 5'-GAT CTT CAT GTC ACG TGG AGT-3'</td>
<td>54</td>
<td>764</td>
<td></td>
</tr>
<tr>
<td>iNOS</td>
<td>Forward: 5'- CCA CAA TAG TAC AAT ACT AC-3', 5'-ACG AGG TGT TCA GCG TGC TC-3'</td>
<td>60</td>
<td>397</td>
<td></td>
</tr>
</tbody>
</table>
The administration of Ang II in higher doses ranging from 6.25 to 40 μg/kg dose-dependently increased the mean 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 μg/kg, dose-dependently reduced 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) inducing 50% reduction in lesion number (50% ID50) was 27 μg/kg. Since the dose of 50 μg/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 compared with the respective values in the intact gastric mucosa. This fall 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 group. The Ang-(1–7)-induced protection and the accompanying rise in the GBF and luminal NO content observed at the 50 μg/kg dose of this peptide were completely reversed by the pretreatment with A-779 (50 μg/kg i.p.) compared with intraperitoneal treatment with Ang-(1–7) (Fig. 2; Table 2).

**Fig. 1.** Mean lesion number and the changes in the GFB in rats pretreated intraperitoneally with vehicle (saline; Veh) or angiotensin II (Ang II) administered intraperitoneally in graded doses ranging from 5 to 40 μg/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 vehicle-controls.

**Fig. 2.** Mean lesion number and the changes in the GFB in rats pretreated intraperitoneally with vehicle-control (saline; Veh) or Ang-(1–7) in graded doses ranging from 6.25 to 50 μg/kg or in those with administration of A-779 (50 μg/kg i.p.), the Mas receptor antagonist, combined with Ang-(1–7). Results are mean ± S.E.M. from eight animals per each experimental group. The groups receiving Ang-(1–7) in graded doses were compared with vehicle-pretreated values for lesion number, GBF, and NO content. The Ang-(1–7) group at the dose of 50 μg/kg, which afforded the maximal protection, was compared with that treated with the combination of Ang-(1–7) at 50 μg/kg with A-779, as indicated under Materials and Methods. Asterisk indicates a significant change (P < 0.05) compared with the respective values of lesion number, GBF, and luminal NO in vehicle-controls. Cross indicates a significant change (P < 0.05) compared to the values obtained in Ang-(1–7) administered intraperitoneally at the dose of 50 μg/kg without the combination with A-779.

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### TABLE 2

<table>
<thead>
<tr>
<th>Type of Test</th>
<th>GBF (ml/min per 100 g)</th>
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<tbody>
<tr>
<td>Intact</td>
<td>46 ± 2.8</td>
</tr>
<tr>
<td>Veh + WRS</td>
<td>27 ± 9.2</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>
</tbody>
</table>
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 seven animals per each experimental group. The values in AVE 0991 group were compared with vehicle- and with those in AVE 0991 group 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 values obtained in AVE 0991-treated rats without concomitant treatment with A-779 treatment.

Fig. 4. Mean lesion number and the alterations in GBF in gastric mucosa pretreated with vehicle, Ang-(1–7), or perindopril with or without NOSynthase inhibitor (L-NNA, 20 mg/kg i.p.) and exposed to WRS. Results are mean ± S.E.M from six animals per each experimental group. The values in Ang-(1–7) or perindopril groups were compared with vehicle (Veh)-controls and with those administered with Ang-(1–7) or perindopril with concurrent treatment with L-NNA. 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 values obtained in rats without L-NNA treatment.

Fig. 5. Mean lesion number and the alteration in GBF in gastric mucosa pretreated with Ang-(1–7) without or with the combination of indomethacin (INDO), SC-560 (SC), and rofecoxib (ROFE) with and without the supplementation with prostaglandin E2 (PGE2). Results are mean ± S.E.M from seven rats per each experimental group. The effect of Ang-(1–7) and inhibitor group were compared with vehicle (Veh)-control group. Asterisk indicates a significant change (P < 0.05) compared with the respective values in vehicle-vehicle group. Double crosses indicate 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.
IL-1β levels were significantly increased in vehicle-pretreated rats exposed to WRS (P < 0.02). Thereafter significant rise in plasma levels of IL-1β and TNF-α was observed in the groups administered with Ang II (50 μg/kg i.p.) compared to those pretreated with vehicle and exposed to WRS (Fig. 7). In contrast, the plasma levels of IL-1β and TNF-α (50 μg/kg i.p.) significantly decreased (P < 0.05) compared to the respective values in Ang-(1–7)-pretreated rats. The alteration in plasma IL-1β and TNF-α mRNAs were significantly upregulated in WRS-induced gastric mucosa, and the ratio of IL-1β or TNF-α mRNA over β-actin mRNA in Ang-(1–7)-pretreated group (Fig. 8, right panel) confirmed that IL-1β and TNF-α mRNAs were significantly upregulated in WRS-induced gastric mucosa. These effects were significantly attenuated in those pretreated with Ang-(1–7) alone (Fig. 8, right panel). In contrast, strong signals for IL-1β and TNF-α mRNAs were observed when rats received the combination of A-779 and Ang-(1–7) compared with those treated with Ang-(1–7) alone (Fig. 7, left panel). The ratio of IL-1β and TNF-α over β-actin confirmed that Ang-(1–7) significantly decreased expression of mRNAs for IL-1β and TNF-α and this effect was reversed in animals administered with the combination of A-779 and 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 mRNA for iNOS was detected as strong signal in gastric mucosa exposed to WRS. In contrast, the strong signal for cNOS mRNA was observed in rats pretreated with Ang-(1–7) (50 μg/kg i.p.) and exposed 30 minutes later to 3.5 hours of WRS compared with those pretreated with vehicle alone. Ratio of cNOS mRNA over β-actin confirmed that cNOS mRNA was significantly increased in Ang-(1–7)-pretreated gastric mucosa over that observed in the vehicle-control gastric mucosa exposed to WRS (Fig. 8, right panel). A weak signal of cNOS mRNA was observed in rats with combined administration of A-779 and Ang-(1–7) alone. Ratio of cNOS mRNA over β-actin confirmed that cNOS mRNA was significantly decreased (P < 0.05) in rats treated with the combination of A-779 and Ang-(1–7) compared with those administered with Ang-(1–7) alone (Fig. 9, right panel).
**WATER IMMERSION AND RESTRAINT STRESS**

![Figure 9](https://www.jpet.aspetjournals.org/doi/figmedia/10.1124/jpet.107.122372/7)

**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 against gastric lesions induced by stress, which is one of the important risk factors for peptic ulcer, hemorrhagic erosions, and microbleeding in animals and humans (Pavel et al., 2008; Konturek et al., 2011). We have shown that parenteral administration of Ang-(1–7) ameliorated dose-dependently the severity of WRS-induced gastric lesions and this effect was accompanied by the increase in GFB and rise in luminal NO content. Blockade of Mas receptor by A-779 inhibited the Ang-(1–7)-induced gastroprotection 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 I 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 inhibitors. We have demonstrated that these 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 and hyperemic actions of Ang-(1–7) and 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 coadministered with Ang-(1–7) 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 (Szilachcic 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., 1993; Kwiecien et al., 2007; Garg et al., 2012). Bregonzio et al. (2009) observed that AT1 blockade led to increase in adrenal corticosterone, reduction in TNF-α and intercellular adhesion molecule 1 (ICAM-1) expression, and neutrophil infiltration in stressed animals. However, the blockade of AT2 receptors does not influence gastroprotective action of corticosterone released during stress (Filarcetowka et al., 1996; Level et al., 2008). Similarly, AT1-receptor antagonists dose-dependently attenuated gastric ulcers and mortality (Merai et al., 2009; Morsy et al., 2009) and counteracted the effects of ischemia and inflammation on the reduction of mucosal neutrophil infiltration and expression of gastric intercellular adhesion molecule 1 and TNF-α (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-(1–9) (Neves et al., 2000; Olszanecki et al., 2009), but this hypothesis requires further studies.

Our results show that WRS increased the expression and plasma levels of TNF-α and IL-1β and that the plasma level of these proinflammatory cytokines was decreased by Ang II, suggesting that the blockade of Ang-(1–7), Ang II, known as a potent vasoconstrictor, aggravates WRS-induced gastric damage due to its proinflammatory action. This is corroborative with the observation that high levels of circulating Ang-(1–7) stimulated the metabolic stress induced by a high-fat diet via decrease in the proinflammatory profile adipose tissue cytokines (Santos et al., 2012). Ang-(1–7) decreased body weight, increased HDL cholesterol, and decreased hepatic expression of COX-2 and IL-1β in abdominal fat of overweight rats (Saavedra et al., 2012). Moreover, Clarricoates et al. (2001) reported a direct binding of Ang-(1–7) to the Ang II receptor, resulting in downregulation 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), whereas the apparent 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., 2003). Enalapril, an inhibitor of ACE, reduced both the plasma 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) acts as an endogenous inhibitor of ACE, enhanced the vasorelaxation of bradykinin (Tom et al., 2003). Our study, therefore, permitted significantly decreased WRS-induced gastric lesions and increased GBF with an extent similar to that observed with Ang-(1–7). t-NNa reduced the gastroprotective and hyperemic activity of perindopril, suggesting that this elevation and rise in the GBF caused by ACE inhibitor might be also mediated by NO. Finally, the luminal content of NO and gastric mucosal expression of mRNA for cNOS were both increased by Ang-(1–7), suggesting that NO derived from cNOS pathway contributes to the beneficial effect of Ang-(1–7) against stress ulcerogenesis. In contrast, the mRNA expression of iNOS was downregulated in these rats, which is consistent with the notion that Ang-(1–7) inhibits WRS lesions due to its potent anti-inflammatory activity.

We clearly demonstrated that Ang-(1–7) significantly and dose-dependently attenuated WRS-induced gastric damage while increasing GBF, and these effects were abolished by d-Ala7-Ang-(1–7) (A-779), the selective antagonist of Mas receptors. Interestingly, the antagonist A-779 has been shown to inhibit most of the physiologic effects of Ang-(1–7) (Santos et al., 2003). Liao et al. (2011) revealed that cardioprotective effect of Ang-(1–7) against ischemia-reperfusion damage is mediated by COX/PG system responsible for the attenuation of malonyldialdehyde content and rise in superoxide dismutase activity. The intestinal mucosal COX-2 expression is regulated by both AT1 and AT2 receptors (Tani et al., 2003). 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 mucosoprotective mechanisms due to an activation of NONOS and COX/PG systems, sensory neuropeptides such as CGRP released from sensory nerves, and the potent inhibition of proinflammatory cytokines and gastric mucosal inflammation.
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). Yusoff et al. (2012) revealed that PGs are important intermediaries 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 gastrophoretic 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 afftert sensory fibers and the major sensory neuropeptide CGRP released from rat sensory nerve endings might play an important role in stress-induced superoxide anion formation and ulcer occurrence. The authors suggest that capsaicin could inhibit expression and release of proinflammatory cytokines and mast cells chymase (CMA1), in gastric inflammation may be regulated by H. pylori associated cytokine. Angiotensin II AT1 receptor antagonism prevent stress.Inflamm. Pathology 41:419–427. 


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