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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Received June 17, 2013; accepted September 18, 2013

ABSTRACT

Angiotensin-(1–7) [Ang-(1–7)] is a major vasoactive metabolite of angiotensin I (Ang I), both being important components of the renin-angiotensin system (RAS). Ang-(1–7) acts via Mas receptor, which was documented in kidneys, heart, brain, and gastrointestinal (GI)-tract. We studied the gastroprotective activity of exogenous Ang-(1–7) in rats exposed to water immersion and restraint stress (WRS) without or with A-779 pretreatment. The WRS lesions were dose-dependently reduced by pre- or post-treatment with Ang-(1–7), which also increased the gastric blood flow (GBF) and renal content of NO. COX-1 and COX-2 inhibitors or L-NNA (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 attenuated the reduction of Ang-(1–7)-induced gastric lesions and rise 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 worsened 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 involves 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, brain, 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 motility and electrolyte homeostasis, maintenance of vascular 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 hyper trophy, 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), enhances the production of reactive oxygen species such as hydroxyl radicals, and increases cell proliferation.

Received June 17, 2013; accepted September 18, 2013.
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 esophagus, gastric, small intestinal, and colonic mucosa (Hirasawa et al., 2002; Casselbranth et al., 2009; Hallersund et al., 2011). 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 axis and the attenuation of vasoconstrictor and proinflammatory activity of Ang II (Pavel et al., 2009; Morsy et al., 2009; Gemici et al., 2010; Saavedra et al., 2010; Santos et al., 2011).

Antagonists of AT₁ receptor candesartan and telmisartan prevented stress-induced gastric lesions (Fregonzio et al., 2003,2004; Merai et al., 2009). Angiotensin-(1–7) [Ang–(1–7)] is a downstream peptide generated from angiotensin I through angiotensin-converting enzyme (ACE) homolog ACE2 or neutral endopeptidase (NEP, also known as neprilysin) 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; Xie et al., 2011). Ang–(1–7) acting via its own G protein-coupled receptor, called Mas (Santos et al., 2006; Stegbauer et al., 2006) exhibit the vasodilatory, antihypertensive, cardioprotective, and antifibrotic effects. Ang–(1–7) is quickly degraded to Ang–(1–7) by plasma kallikrein and renin. 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 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 carried out in accordance with the guidelines of the Helsinki Declaration regarding the handling and experimental use of animals.

Stress-Induced Gastric Lesions, Chemicals, and Drugs

Application. To induce gastric lesions, rats were immobilized in individual boltman cages and immersed in the cold water (23°C) for 3.5 hours of WRS (Brzozowski et al., 2000, Konturek et al., 2001). Animals in the 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: A) exogenous Ang–(1–7) (6.25–50 μg/kg, i.p.); B) Ang–(1–7) Mas receptor antagonist (Bayorh et al., 1999; Santos et al., 2006, 2007) or perindopril (5 mg/kg i.p.), the selective Ang–(1–7) Mas receptor antagonist (Bayorh et al., 1999; Santos et al., 2006, 2007) with or without the combination with Ang–(1–7) or perindopril 5 mg/kg i.p.); and C) perindopril (5 mg/kg, i.p.), the selective Ang–(1–7) receptor antagonist (Bayorh et al., 1999; Santos et al., 2006, 2007) with or without the combination with Ang–(1–7) or perindopril 5 mg/kg i.p. The nonpeptide Ang–(1–7) receptor agonist (Pinheiro SV et al., 2004; Santos and Fereira, 2006), respectively.

In series A–C, the effects of cotreatment with Ang–(1–7) or perindopril, with or without the combination with i-NNA (20 mg/kg i.p.), the competitive inhibitor of NO-synthase activity, on WRS lesions and stress in the G were determined.

The involvement of endogenous PG in the gastroprotective effects of exogenously 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 i.g.) 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 Labchemikalien (Schelldorf, Germany) except of SC-560 and rofecoxib purchased from Cayman.
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 H2-gas 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-1 and TNF-α as described previously (Kwiecien et al., 2012b). In brief, the plasma 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 plasma sample (50 μl) was incubated with biotinylated antibodies specific to rat TNF-α and IL-1β, washed three times with assay buffer, and finally conjugated with streptavidin peroxidase to form a complex with a stabilized chromogen as described elsewhere (Kwiecien et al., 2012b).

Table 1: The annealing temperature, nucleotide sequence primers, and size of products used for RT-PCR determination

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence</th>
<th>Annealing Temperature</th>
<th>Size of PCR Product</th>
</tr>
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<tbody>
<tr>
<td>c-NOS</td>
<td>Forward: 5'-TAC CGG ACA GCA AAT CCA C-3', Reverse: 5'-CAG GCT GCA GTC CTT TGA TC-3'</td>
<td>63.5 °C</td>
<td>540 bp</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Forward: 5'-GCT ACC TAT GTC TGG CCC GT-3', Reverse: 5'-GAC CAT TGC TGT TCT CTA GG-3'</td>
<td>62 °C</td>
<td>543 bp</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Forward: 5'-TAC TGA ACT TCG GGG TGA TTG GTC C-3', Reverse: 5'-CAG CCT TGT CCC TTT AAG AGA ACC-3'</td>
<td>56 °C</td>
<td>295 bp</td>
</tr>
<tr>
<td>β-Actin</td>
<td>Forward: 5'-TTG TAA CCA ACT GGG AGG ATA TTG-3', Reverse: 5'-GAT CTT CAT GCT TAT GCT AGG-3'</td>
<td>54 °C</td>
<td>764 bp</td>
</tr>
<tr>
<td>iNOS</td>
<td>Forward: 5'-CCA CAA TAG TAC AAT ACT AC-3', Reverse: 5'-ACG AGG TGT TCA GCG TGC TC-3'</td>
<td>60 °C</td>
<td>397 bp</td>
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Statistical Analysis. Results of each 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 GFB and in absolute values for lesion number. The control rats did not differ from experimental rats in terms of relevant characteristics, such as source of primiparous breeder, age, weight, diet, and housing conditions. There was no initial pairing of animals, the paired statistical tests were not used.

Results

Mean Lesion Number and GFB 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 GFB (Fig. 1). The pretreatment with Ang II applied in a dose of 5 μg/kg failed to significantly affect the mean lesion number and GFB compared with vehicle-control.

Table 1: The annealing temperature, nucleotide sequence primers, and size of products used for RT-PCR determination.
The administration of Ang II in higher doses ranging from 10 to 40 μg/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 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) inhibiting WRS lesions by 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 (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, pretreatment with Ang-(1–7) resulted in a significant increase in the GBF (P < 0.05) compared with the pretreatment with saline. The Ang-(1–7)-induced protection of the accompanying rise in the GBF and luminal NO content observed at the 50 μg/kg dose of this peptide was completely reversed by the pretreatment with A-779 (50 μg/kg, i.p.) compared with intraperitoneal saline with Ang-(1–7) (Fig. 2; Table 2).

Effect of AVE 0991, the Agonists of Ang-(1–7) Mas Receptor, on WRS-Induced Gastric Lesions and Alterations in the GBF. As shown in Fig. 3, pretreatment with AVE 0991 (50 μg/kg i.p.) significantly reduced the mean lesion number (P < 0.05) and produced a significant increase in the GBF (P < 0.05) compared with the respective values in vehicle-control pretreated rats. These decreases in lesion number and an increase in GBF induced by AVE 0991 were completely reversed by the combination of Ang-(1–7) 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 μg/kg i.p.) significantly reduced the WRS-induced gastric lesions and increased GBF, with the effect similar to the respective values presented in Fig. 1. The pretreatment with perindopril (5 mg/kg i.p.) also significantly decreased the mean WRS-induced gastric lesions (P < 0.05), and increased GBF compared to vehicle-control. Administration of L-NNA (20 mg/kg i.p.), which itself failed to significantly affect the lesion number and GBF compared to vehicle-treated control, reversed the protective action 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 Gastroprotection against WRS-Induced Gastric Damage and Alteration in GBF. As shown in Fig. 5, the pretreatment with Ang-(1–7) (50 μg/kg i.p.) caused a similar decrease in the mean number of WRS-induced lesions (P < 0.05). 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 (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, pretreatment with Ang-(1–7) resulted in a significant increase in the GBF (P < 0.05) compared with the pretreatment with saline. The Ang-(1–7)-induced protection of the accompanying rise in the GBF and luminal NO content observed at the 50 μg/kg dose of this peptide was completely reversed by the pretreatment with A-779 (50 μg/kg, i.p.) compared with intraperitoneal saline with Ang-(1–7) (Fig. 2; Table 2).

Table 2: Effects of Pretreatment on GBF

<table>
<thead>
<tr>
<th>Type of Test</th>
<th>GBF (ml/min per 100 g)</th>
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<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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</table>

Results are mean ± S.E.M. from seven animals per each experimental group. Ang-(1–7) and perindopril significantly reduced the WRS-induced gastric lesions and increased GBF, with the effect similar to the respective values presented in Fig. 1. The pretreatment with perindopril (5 mg/kg i.p.) also significantly decreased the mean WRS-induced gastric lesions (P < 0.05), and increased GBF compared to vehicle-control. Administration of L-NNA (20 mg/kg i.p.), which itself failed to significantly affect the lesion number and GBF compared to vehicle-treated control, reversed the protective action in lesion number and the rise in GBF evoked by Ang-(1–7) or perindopril (Fig. 4).
gastric lesions accompanied by a significant increase in the GBF as presented in Fig. 2. The pretreatment with COX-1 and COX-2 inhibitors alone significantly reduced the mean lesion number and produced a significant increase in GBF 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 significantly attenuated by pretreatment with 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) treatment on lesion number and GBF was compared with that achieved with COX-1 and COX-2 inhibitors in the absence or the presence of PGE2. Asterisk indicates a significant change (P < 0.05) compared with the respective value in vehicle-high group. Cross indicates significant a 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.

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 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) and with PGE2.

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
cNOS mRNA was significantly decreased (Fig. 8, right panel). A weak signal of cNOS mRNA was detected in Ang-(1–7) pretreated with vehicle (Fig. 8). The ratio of cNOS mRNA over α-actin confirmed that mRNA for iNOS was negligible in the intact gastric mucosa, but expression observed in Ang-(1–7)-pretreated animals was reversed in those concomitantly treated with A-779. The ratio of cNOS mRNA over α-actin confirmed that mRNA for iNOS 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. 8, right panel).

AngII and TNF-α mRNAs were significantly upregulated in WRS-induced gastric mucosa. These effects were significantly attenuated in those pretreated with Ang-(1–7) (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, and this effect was significantly decreased 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) (Fig. 9, lower panel). The decrease in iNOS mRNA expression observed in Ang-(1–7)-pretreated animals was reversed in those concomitantly treated with A-779. The ratio of iNOS mRNA over β-actin confirmed that mRNA for iNOS was significantly increased when A-779 was combined with Ang-(1–7) (Fig. 9, lower panel).
Fig. 8. Determination of cNOS mRNA, IL-1β, and TNF-α expression by RT-PCR (left panel) and the ratio of cNOS, IL-1β, and TNF-α mRNAs over β-actin mRNA (right panel) in the vehicle (Veh)-control gastric mucosa (lane 1) and in those pretreated with Ang-(1–7) (50 μg/kg i.p.) (lane 2), and A-779 (50 μg/kg i.p.) coadministered with Ang-(1–7) (50 μg/kg i.p.) (lane 3) and exposed to WRS for 3.5 hours; M, DNA size marker. Mean ± S.E.M. of four determinations in four rats per group. Analysis of the values of the ratio of cNOS, IL-1β, and TNF-α mRNAs expression vs. β-actin mRNA was performed between values in Ang-(1–7)-pretreated and in those pretreated with combination of A-779 and Ang-(1–7) versus Ang-(1–7) alone. Asterisk indicates a significant change (P < 0.05) compared with vehicle-control gastric mucosa. Cross indicates a significant change (P < 0.05) compared with Ang-(1–7) alone.

Fig. 9. Determination of NOS expression by RT-PCR (left panel) and the ratio of iNOS mRNA over β-actin mRNA (right panel) in the intact gastric mucosa (lane 1), vehicle (Veh)-control gastric mucosa exposed to WRS (lane 2), and in those pretreated intraperitoneally with Ang-(1–7) (50 μg/kg i.p.) (lane 3), and A-779 (50 μg/kg i.p.) combined with Ang-(1–7) (50 μg/kg i.p.) (lane 4) and exposed to WRS for 3.5 hours; M, DNA size marker. Mean ± S.E.M. of four determinations in four rats per group. Analysis of the values of the ratio of iNOS mRNA expression in gastric mucosa was performed between values in Ang-(1–7)-pretreated and vehicle-pretreated and in those treated with combination of A-779 and Ang-(1–7) versus Ang-(1–7) alone. Asterisk indicates a significant change (P < 0.05) compared with vehicle-control gastric mucosa. Cross indicates a significant change (P < 0.05) compared with Ang-(1–7) alone.

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 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 cGMP and rise in 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 I metabolite because this protection and an increase in GBF 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 iNOS was upregulated while expression of cNOS, 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 GBF 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 (Szilachcie 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; Konturek et al., 2011; Pavel et al., 2008). Bregonzio et al. (2004) observed the reduction of sympathetic neural activity and by attenuating cytokines and neutrophil infiltration in stressed animals. However, the blockade of AT1 receptors does not exclude that the beneficial effect of AT1-receptor antagonists may also be mediated by NO. Finally, the luminal content of NO was increased by Ang-(1–7) and gastric mucosal expression of mRNA for cNOS was both increased by Ang-(1–7), suggesting that NO derived from endogenous Ang II could contribute to pathogenesis of cold-restraint stress ulcer in obstructive jaundice rats (Mou et al., 1998). Furthermore, Ang-(1–7) acts as an endogenous inhibitor of ACE, reduced both the gastric and gastric mucosal Ang II level, decreased gastric blood flow, and increased the extent of mucosal damage (Mou et al., 1998). Enalapril, an inhibitor of ACE, reduced both the gastric and gastric mucosal Ang II level, decreased gastric blood flow, and increased the extent of mucosal damage (Mou et al., 1998). However, Ang-(1–7) attenuated gastric ulcer in rats pretreated with Ang-(1–7), suggesting that this difference between Ang-(1–7) and Ang II with respect to proinflammatory cytokines. Moreover, the endogenous Ang II could contribute to the selective action of bradykinin (Tom et al., 2003). In our study, perindopril significantly decreased WRS-induced gastric lesions and raised GBF with an extent similar to that of Ang-(1–7). NOS is known to 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 NO system 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 AT1 receptors. Interestingly, the antagonist A-779 has been shown to inhibit both the physiological effects 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.

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 mucusoprotective 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 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 alleviated by Ang II, suggesting that AT1-receptor antagonists could have a potent vasoactive effect on proinflammatory cytokines. In contrast to Ang-(1–7), the selective antagonist Ang-(1–7), known as a potent vasoactive 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., 1998). Furthermore, Ang-(1–7) increases the expression of iNOS (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.

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 mucusoprotective 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 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.
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). Yousif 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 gastropathy 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) are markedly impaired in rats with capsaicin-induced functional denervation of sensory fibers. This indicates that besides NO and PG afferent sensory fibers and the major sensory neuropeptides such as CGRP. In contrast to Ang II, Ang-(1-7) potentiates responses to bradykinin but does not change responses to angiotensin II receptor and local renin-angiotensin system.

Authorship Contributions

Conducted experiments: Magierowski, Kwiecien, Brzozowski.
Conducted the data analysis: Krzyzsi-Maczka, Olzanski, Korbutowicz.
Performed data analysis: Maszewski, Jasnos, Brzozowski.
Wrote or contributed to the writing of the manuscript: Magierowski, Kwiecien, Brzozowski.

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