Anti-inflammatory effects of angiotensin II AT₁ receptor antagonism prevent stress-induced gastric injury

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taneously hypertensive rats were pretreated for 14 days with the AT₁ receptor antagonist candesartan before cold-restraint stress. AT₁ receptors were localized in the endothelium of arteries in the gastric mucosa and in all gastric layers. AT₁ blockade increased gastric blood flow by 40–50%, prevented gastric ulcer formation by 70–80% after cold-restraint stress, reduced the increase in adrenomedullary epinephrine and tyrosine hydroxylase mRNA without preventing the stress-induced increase in adrenal corticoste-
rone, decreased the stress-induced expression of TNF-α and that of the adhesion protein ICAM-1 in arterial endothelium, decreased the neutrophil infiltration in the gastric mucosa, and decreased the gastric content of PGE₂. AT₁ receptor blockers prevent stress-induced ulcerations by a combination of gastric blood flow protection, decreased sympa-
thisoadrenal activation, and anti-inflammatory effects (with reduction in TNF-α and ICAM-1 expression leading to reduced neutrophil infiltration) while maintaining the protective glucocorticoid effects and PGE₂ release. Angiotensin II has a crucial role, through stimulation of AT₁ receptors, in the production and progression of stress-induced gastric injury, and AT₁ receptor antagonists could be of therapeutic benefit.

STRESS INDUCES ACUTE GASTRIC mucosal lesions (33) by complex psychological factors influencing individual vulnerability, stimulation of specific brain pathways regulating autonomic function, decreased blood flow to the mucosa, increase in muscular contractility, mast cell degranulation, leukocyte activation, and increased free radical generation resulting in increased lipid per-
oxidation (2, 33, 49, 55).

Cold-restraint stress is a commonly used and clini-
cally relevant experimental model for acute gastric damage (44). A sudden blood flow reduction to the gastric mucosa and increased free radical formation play fundamental roles in ulcer production (49). Main-
tenance of gastric blood flow is important to protect the mucosa from endogenous and exogenous damage factors.

Angiotensin II (ANG II) is a stress hormone (40), the levels of which dramatically increase in plasma and tissues, including stomach, during stress (10, 54). ANG II not only regulates vascular tone in resistance arter-
ies (16) and in the brain (31) but also constricts the gastric vasculature through AT₁ receptor stimulation (19). In addition, ANG II generates reactive oxygen species with cellular damage and inflammation (36). The mucosal vasoconstriction and proinflammatory ef-
effects of ANG II could contribute to the production of stress-induced gastric ulcers.

Inhibition of ANG II AT₁ receptors with peripheral and central receptor antagonists (31) prevents the sympa-thoadrenal and hypothalamic-pituitary-adrenal response to isolation stress (4) and protects the brain from injury by reducing the cerebral blood flow decrease during stroke (21, 32). We considered whether a similar treatment could reduce the incidence of stress-
induced acute gastric ulcers. We studied a widely used strain of spontaneously hypertensive rats (SHR) char-
acterized by increased sympatheticadrenal reactivity to stress (28, 51) because of the well-known association between stress and cardiovascular disease (12).

To determine whether AT₁ receptor blockade could be of therapeutic advantage in stress-related disorders, we pretreated SHR with an AT₁ receptor blocker before cold-restraint stress. We tested whether any protective effect of AT₁ antagonists could be related to a combi-
nation of inhibition of the stress-induced hormone and sympa-thoadrenal response, inhibition of the vasoconstric-
tor effects of ANG II in the gastric blood flow, and inhibition of mucosal inflammation.

MATERIALS AND METHODS

Animals. Adult, 8-wk-old male SHR weighing 200–250 g were purchased from Taconic Farms (Germantown, NY), housed at 22°C under a 12:12-h light-dark cycle, and given free access to normal rat diet and tap water. The National
Institute of Mental Health Animal Care and Use Committee approved all procedures.

First experiment: localization of AT1 receptors in gastric blood vessels and effects of treatment with an AT1 receptor antagonist on gastric blood flow. Groups of six or seven rats were implanted subcutaneously with osmotic minipumps the day after the arrival of the animal facility to deliver the ANG II AT1 receptor antagonist candesartan (1 mg/kg per day) or vehicle (0.1 N Na2CO3) at a constant infusion rate for 14 days. These animals were not submitted to cold-restraint stress. Different groups of rats were used for estimation of gastric blood flow after treatment and AT1 receptor immunohistochemical localization.

Second experiment: effects of pretreatment with an AT1 receptor antagonist on cold restraint-induced ulcers. Different groups of six or seven animals were used to study the number of gastric ulcers after cold-restraint stress or for autoradiography, biochemical, or immunohistochemical analysis. Animals treated with vehicle or candesartan were submitted to stress between 9:00 AM and 12:00 PM. Animals were fasted for 24 h before the experiment with free access to water. After 4 h, gastric restraining devices, and maintained at 4°C for 2 h (44). Immediately after the end of the restraining period, the animals were killed by decapitation. Additional groups of six animals treated with vehicle or candesartan were used as controls and remained in their cages at 22°C until killed by decapitation after a 24-h period of fasting with free access to water (nonstressed rats).

Third experiment: effects of oral pretreatment with an AT1 receptor antagonist. Groups of six animals received oral candesartan-cilexetil (TCV-116; Astra, Mölndal, Sweden) at 10 mg/kg per day for 14 days or received vehicle, both dissolved in their drinking water. Candesartan-cilexetil was first dissolved as a 1 mg/ml stock solution in polyethylene glycol 400/ethanol/Cremophor EL (Sigma)/water (10/5/2/83%), adjusted to pH 9 with 0.2 M Na2CO3. The solution was diluted in water to a final concentration ≤1/0.5/0.2% polyethylene glycol 400/ethanol/Cremophor EL. We evaluated the number of stress-induced ulcers, hormones, catecholamines, and tyrosine hydroxylase (TH) mRNA.

Tissue preparation. The pituitary and adrenal glands were immediately removed, frozen in isopentane at −30°C on dry ice, and stored at −80°C until assay. The stomachs were immediately removed and opened along the greater curvature, the lumen was rinsed with saline, and the mucosa of the glandular portion was examined macroscopically. Positive lesions were identified in 2-cm2 areas by using a magnifying glass and were defined as erosions of at least 1-mm diameter with sharply demarcated edges and black or red bases.

For receptor binding, stomach sections (16 μm thick) were cut in a cryostat at −20°C, thaw-mounted on poly-L-lysine-coated slides (Labscientific, Livingston, NJ), dried overnight in a desiccator at 4°C, and stored at −80°C until assay. For in situ hybridization, adrenal sections were cut as above and stored at −80°C until assay.

Measurement of gastric blood flow. We used groups of six animals treated with vehicle or candesartan as described above and not submitted to stress. Animals were fasted for 24 h, followed by anesthesia with sodium pentobarbital (50 mg/kg ip; Nembutal, Abbot Laboratories, Chicago, IL). Gastric blood flow was determined by injecting, through a catheter in the left atrium, 500 μl of a suspension containing 1.3 × 106 microspheres (15 μm each; Red BioPAL; BioPAL, Worcester, MA) in a solution of saline with 0.05% Tween 80 and 0.01% Thimerosal. A reference blood sample was simultaneously drawn from a femoral arterial catheter using a withdrawal pump to calculate absolute regional blood flow (38). Immediately after, the animals were killed by decapitation, and their stomachs were removed and rinsed with a solution of a saline substitute (Sanssaline; BioPAL) that lowers sodium and chloride levels. Blood flow was determined separately in the glandular portion and fundus, after these regions were identified as described (8), and were isolated immediately after tissue removal. Blood flow for the whole stomach was calculated from the results of the glandular portion and fundus. Blood and tissue samples were dried overnight at 70°C and sent for analysis to BioPAL. The samples were activated by exposure to a field of neutrons, the activated vials were stored for 48 h to allow decay of short-lived activation products, and after this decay period spectrophotographic analysis was performed to measure the concentration of the remaining radioactive nuclei in each sample (38). The microsphere concentration of each segment [disintegrations per minute (dpm)/g] was normalized to the microsphere concentration measured in the 2-min reference blood collection (in dpm·min−1·ml−1) to obtain blood flow (ml·min−1·g−1) (38).

Hormone and catecholamine determinations. Pituitary and adrenal hormones were determined by radioimmunoassay using commercially available kits (ICN Biomedicals, Costa Mesa, CA) (4). Adrenal catecholamines were measured by reverse-phase HPLC with electrochemical detection as described (4).

Determination of PGE2 content. We homogenized the tissue in PBS (pH 7.4) containing 200 μM indomethacin and determined the PGE2 content by RIA with a commercially available kit (Biotrack; Amersham Pharmacia Biotech, Piscataway, NJ).

ANG II receptor binding. We incubated adjacent sections of the glandular part of the stomach with 0.5 nM [125I]sar-cosine-1-ANG II ([125I]Sar1-ANG II) from Peninsula Laboratories, iodinated by the Peptide Radiiodination Service Center (Washington State University, Pullman, WA) (total binding) or with [125I]Sar1-ANG II in the presence of 10−6 M unlabeled ANG II (Peninsula) (nonspecific binding) as described (50). Specific binding to ANG II receptors was the difference between total binding and nonspecific binding. Binding for AT1 receptors was determined by incubating consecutive sections with the addition of 10−6 M losartan (DuPont-Merck, Wilmington, DE) and the binding to [125I]Sar1-ANG II was displaced by the selective AT1 receptor antagonist. Binding for AT2 receptors was determined by incubating consecutive sections with 10−6 M PD-123319 (Parke-Davis, Ann Arbor, MI), and the binding to [125I]Sar1-ANG II was displaced by the selective AT2 receptor antagonist (50). Sections were exposed to BioMax MR films (Kodak, Rochester, NY) together with 14C microscales (American Radiolabeled Chemicals, St. Louis, MO). Optical densities of autoradiograms were quantified as described (29, 50).

In situ hybridization of TH mRNA. One antisense oligonucleotide of 48-mer for the rat TH cDNA sequence was synthesized (Lofstrand Labs). The oligonucleotide was localized in nt 1562–1609 (17). Labeling was performed with a 3′-end labeling kit (Amersham) using terminal deoxynucleotidyl transferase to a specific activity of 3–4 × 107 dpm/μg. Each reaction was performed with 70 pmol of oligonucleotide in the presence of 70 μCi of [α-35S]ATP (1500 Ci/m mole) (Amersham). The labeled oligonucleotides were separated from unincorporated nucleotides using MicroSpin G-25 columns (Amersham). Consecutive rat adrenal sections were incubated with labeled antisense oligonucleotide or with labeled oligonucleotide with excess unlabeled probe (57 pmol/ml) as described by Wisden and Morris (53).
**RESULTS**

**Localization of AT$_1$ receptors by immunocytochemistry.** In vehicle-treated nonstressed rats, AT$_1$ receptors were expressed in all vessels studied, including the endothelium of arteries located in the mucosa of the gastric glandular portion (Fig. 1A), the endothelium of arteries located in the muscularis mucosa, larger arteries in the submucosa, and large veins in the submucosa (not shown).

**Gastric blood flow.** Pretreatment for 14 days with the AT$_1$ antagonist significantly increased the blood flow in the glandular portion and in the whole stomach. In the fundus, the increase in the blood flow after AT$_1$ blockade was substantial (~30%), but the change was not statistically significant (Fig. 1B).

**Stomach lesions.** Cold-restraint stress produced large numbers of mucosal lesions in the glandular portion of the stomach of rats treated with vehicle (Fig. 2A). Pretreatment for 14 days with the AT$_1$ antagonist administered subcutaneously reduced the number of stress-induced gastric ulcers by 80% (Fig. 2B). The number of lesions observed in stressed animals pretreated with the AT$_1$ blocker was not different from that observed in nonstressed controls (Fig. 2B). In nonstressed rats, there was an occasional stomach lesion, in a concentration of <1 lesion/cm$^2$, after either vehicle treatment or AT$_1$ receptor blockade (Fig. 2B).

The AT$_1$ antagonist for 14 days also significantly reduced the number of stress-induced lesions by ~70% (Fig. 2B).

**Hormone and catecholamine responses to stress.** Cold-restraint stress significantly increased adrenal corticosterone content but did not affect the content of ACTH in the pituitary gland of vehicle-treated rats (Table 1). Pretreatment with the AT$_1$ antagonist had no effect on adrenal corticosterone content in unstressed animals, did not modify the corticosterone response to cold restraint, and did not significantly change the pituitary ACTH content (Table 1).

Stress significantly decreased the adrenal content of epinephrine, with no modification in norepinephrine levels, and significantly increased transcription of the rate-limiting enzyme in catecholamine synthesis, TH, as evidenced by increased expression of TH mRNA in the adrenal medulla (Table 1). Pretreatment with the AT$_1$ antagonist did not modify the adrenal epinephrine or norepinephrine content or the expression of TH mRNA in nonstressed controls. However, pretreatment with the AT$_1$ antagonist significantly diminished the increase in adrenomedullary TH mRNA and the reduction in adrenal epinephrine content produced by stress, without significantly modifying the adrenal norepinephrine concentration (Table 1).
Pretreatment with oral candesartan produced changes similar to those obtained after subcutaneous administration of the AT1 antagonist, namely reduction of the decrease in adrenal epinephrine content and reduction of the increase in adrenomedullary TH mRNA observed during stress, without alterations in the stress-induced increase in adrenal corticosterone levels (results not shown).

ANG II AT1 and AT2 receptor binding. In control, nonstressed rats, we detected low levels of AT1 receptors in the mucosa, muscularis mucosa, and muscle of the glandular portion of the stomach. The muscularis mucosa expressed a higher level of AT1 receptors than the mucosa or muscle layers (Fig. 3). In vehicle-treated rats, cold-restraint stress produced a total disappearance of AT1 receptor binding in the mucosa and a massive decrease (−95%) in AT1 receptor binding in the muscularis mucosa (Fig. 3). Conversely, no alteration in AT1 receptor binding was noted after stress in the muscle layer (Fig. 3).

As expected, administration of the AT1 receptor antagonist to nonstressed rats decreased AT1 receptor binding in the three layers studied, and the decrease in receptor binding was significant in the muscularis mucosa and muscle layers (Fig. 3). In stressed rats pretreated with the AT1 receptor antagonist, there was no

Table 1. Corticosterone, ACTH, catecholamines, and tyrosine hydroxylase mRNA after cold restraint and AT1 receptor blockade

<table>
<thead>
<tr>
<th></th>
<th>Nonstress Vehicle</th>
<th>Nonstress AT1 Blockade</th>
<th>Stress Vehicle</th>
<th>Stress AT1 Blockade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal corticosterone, ng/adrenal</td>
<td>1,838 ± 224</td>
<td>1,705 ± 194</td>
<td>3,280 ± 183*</td>
<td>3,408 ± 303*</td>
</tr>
<tr>
<td>Pituitary ACTH, ng/gland</td>
<td>48 ± 5</td>
<td>33 ± 9</td>
<td>49 ± 10</td>
<td>37 ± 10</td>
</tr>
<tr>
<td>Adrenal epinephrine, ng/adrenal</td>
<td>10,016 ± 383</td>
<td>9,138 ± 649</td>
<td>8,189 ± 692*</td>
<td>8,883 ± 268</td>
</tr>
<tr>
<td>Adrenal norepinephrine, ng/adrenal</td>
<td>2,506 ± 235</td>
<td>2,695 ± 376</td>
<td>2,218 ± 239</td>
<td>2,570 ± 213</td>
</tr>
<tr>
<td>Adrenal TH mRNA, nCi/mg</td>
<td>2.1 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>4.8 ± 0.1*</td>
<td>3.6 ± 0.4*</td>
</tr>
</tbody>
</table>

Results are presented as mean ± SE of groups of six animals, measured individually. Animals were treated with vehicle or with candesartan, 1 mg·kg⁻¹·day⁻¹ sc, as described in MATERIALS AND METHODS. TH, tyrosine hydroxylase. *P < 0.05 vs. nonstressed rats treated with vehicle. †P < 0.05 vs. stressed rats treated with vehicle.
detectable AT₁ binding in the mucosa or in the muscle layer (Fig. 3). In the muscularis mucosa, AT₁ binding in candesartan-treated rats submitted to stress was similar to that present in candesartan-treated nonstressed rats (Fig. 3).

In control, nonstressed rats treated with vehicle, AT₂ receptor expression was lower than that of AT₁ receptors in the three layers of the glandular portion of the stomach. In vehicle-treated rats, stress completely suppressed AT₂ receptor expression in the mucosa but did not alter the low AT₂ receptor expression in the muscularis mucosa and muscle layers (Fig. 3). AT₁ receptor blockade eliminated AT₂ receptor binding in the mucosal layer of nonstressed rats without affecting the AT₂ binding expression in the muscularis mucosa or muscle layers (Fig. 3). Pretreatment with the AT₁ receptor antagonist did not modify the response of AT₂ receptor expression to stress, with exception of increased AT₂ receptor binding in the muscle layer (Fig. 3). In summary, the glandular portion of the stomach expressed higher AT₁ than AT₂ receptor numbers, receptor expression was higher in the muscularis mucosa, both stress and AT₁ blockade decreased AT₁ receptor binding, and the effects of either stress or AT₁ blockade on AT₂ receptors was not substantial.

**TNF-α immunocytochemistry.** In the mucosa of the glandular portion of the stomach, the expression of TNF-α was low in control, nonstressed rats treated with vehicle (Fig. 4A) and in nonstressed animals treated with the AT₁ blocker (not shown). Stress produced a massive increase in TNF-α immunoreactivity localized to cells infiltrating the gastric mucosa and...
localized to the interstitial tissue (Fig. 4B). This increase was completely prevented by pretreatment with the AT1 receptor antagonist (Fig. 4C). Cells positive for TNF-α revealed a morphology compatible with that of infiltrating neutrophils.

**ICAM-1 immunocytochemistry.** We detected ICAM-1 expression in the endothelium of small arteries located in the mucosa, larger arteries in the submucosa, and venules in the submucosa of the glandular portion of the stomach (Fig. 5). Although ICAM-1 expression was very low in all vessels from nonstressed rats (not shown), endothelial ICAM-1 expression markedly increased and was very intense in all vessels from stressed animals pretreated with vehicle (Fig. 5, A, C, and E). AT1 blockade very substantially decreased ICAM-1 expression in all vessels from rats submitted to stress (Fig. 5, B, D, and F) to levels apparently not different from those in untreated nonstressed controls and completely abolished the low expression of ICAM-1 in endothelial cells of control, nonstressed rats (not shown).

**Neutrophil infiltration.** Large numbers of infiltrating neutrophils were detected by immunocytochemistry and hematoxylin and eosin in animals submitted to stress and treated with vehicle (Fig. 6, A, C, and D). Neutrophils were present around blood vessels and in the interstitial space of the gastric mucosa (Fig. 6D), and they were also present in large numbers in the gastric lesions (Fig. 6C). Pretreatment with the AT1 antagonist markedly reduced the number of neutrophils in the stressed group (Fig. 6E). Quantification revealed a large decrease in neutrophil numbers when stressed rats were pretreated with the AT1 receptor antagonist (Fig. 6F). Some neutrophils were detected in nonstressed animals treated with vehicle, and their number was decreased after treatment with the AT1 blocker (not shown).

**PGE2 content in the stomach.** We studied the content of PGE2 in the whole stomach. The PGE2 content was very significantly decreased by both cold-restraint stress in vehicle-treated rats (50% reduction) or by AT1 antagonism in control, nonstressed animals (~70% reduction) (Fig. 7). In animals pretreated with the AT1 antagonist, stress further decreased PGE2 content to a level ~20% of that of vehicle-treated nonstressed controls (Fig. 7). The decrease in PGE2 content produced by stress or by AT1 antagonism, however, was not statistically different when compared between the vehicle-treated, stressed group and the groups treated with candesartan (Fig. 7).

**DISCUSSION**

Our results demonstrate that pretreatment with an ANG II AT1 receptor antagonist dramatically decreases the number of ulcerations produced by cold-restraint stress, protecting the gastric mucosa from stress-induced injury, in a model of genetically hypertensive rats hypersensitive to stress (28, 51). ANG II appears to be an essential factor in the production of stress-induced gastric ulcers, and selective blockade of AT1 receptors may have therapeutical advantages in these conditions.

We found several mechanisms, probably interrelated, that could be involved in the protective effect of the AT1 antagonist. First, blockade of AT1 receptors modified the adrenomedullary response to stress. During intense acute stress, such as immobilization, there is an initial massive release of adrenomedullary catecholamines, followed by a compensatory increase in catecholamine synthesis, with fast enhancement in transcription of the rate-limiting enzyme TH (25). However, because of the initial massive epinephrine release, the net result is an early decrease in adrenal epinephrine content (25, 48). Confirming these reports, we have found both increased TH mRNA expression, an index of increased transcription, and decreased epinephrine content, an index of increased release, in our stress model. Pretreatment with the AT1 receptor antagonist partially inhibited the stress-induced increase in epinephrine content (25, 48). Confirming these reports, we have found both increased TH mRNA expression, an index of increased transcription, and decreased epinephrine content, an index of increased release, in our stress model. Pretreatment with the AT1 receptor antagonist partially inhibited the stress-induced increase in epinephrine content (25, 48).
in TH mRNA expression and the decrease in adrenal epinephrine content. Thus our results indicate that pretreatment with an AT1 receptor antagonist reduced both the stress-induced increase in epinephrine synthesis and in epinephrine release, as reported earlier in a model of isolation stress (4). Blunting the response of the sympathoadrenal system during stress could have contributed to decreased gastric vasoconstriction. The degree of inhibition of adrenal catecholamine synthesis by the AT1 antagonist during cold-restraint stress was smaller than that observed during isolation stress (4). These differences were probably related to the intensity of the stress in the present study, clearly more intense than that of isolation in individual cages.

Second, AT1 receptor blockade did not prevent the increase in adrenal corticosterone produced by cold-restraint stress. This is of interest because a similar treatment completely blocked the hypothalamic-pituitary-adrenal hormonal response to isolation (4) and demonstrates that ANG II regulates the stress reaction differently depending on the kind and intensity of the stress (22). Because maintenance of a normal pituitary-adrenal response to stress parallels an almost total protection from gastric ulceration, our results support the hypothesis that, although large doses of exogenously administered corticosteroids are ulcerogenic, endogenous corticoids actually contribute to protect the gastric mucosa from ulceration during stress, probably by contributing to an increase in blood flow and secretion of bicarbonate (13).

Third, the blood flow to the stomach was significantly increased in animals treated with the AT1 receptor antagonist compared with vehicle-treated controls before submission to cold restraint, indicating that inhibition of basal ANG II vasoconstrictor tone is an important regulator of gastric blood flow. Although
technical limitations prevented us from determination of the gastric blood flow during the stress procedure, it is reasonable to speculate that blockade of the ANG II system could reduce the decrease in gastric blood flow during stress in a manner similar to that demonstrated during brain ischemia (21, 32). An earlier report negated a role of ANG II AT1 receptors in the gastric vascular perturbances during hemorrhagic shock (19). However, our results demonstrate that ANG II-mediated constriction of the gastric vasculature during stress is a very important mechanism for ulcer production. This hypothesis is supported by the finding that inhibition of ANG II formation with angiotensin-converting enzyme inhibitors correlates with protection of gastric blood flow and decreased ulcer formation in rats with obstructive jaundice (10). The effects of AT1 antagonists on blood flow are probably mediated by receptors localized to the endothelium of arteries located in the gastric mucosa, muscularis mucosa, and submucosa, as demonstrated here.

Fourth, we found AT1 receptors, and lower numbers of AT2 receptors, by autoradiography in all layers of the stomach. These results are similar to those reported earlier in the intestine (43). We report decreased AT1 receptor binding after treatment with the AT1 receptor inhibitor. This was expected, and it is probably a result of receptor occupancy with the insurmountable antagonist candesartan or receptor downregulation (31). The decreased number of gastric AT1 receptors after stress may be dependent on receptor occupancy by increased ANG II levels or receptor downregulation as a compensatory mechanism to increased ANG II stimulation (4).

Fifth, we have confirmed that cold restraint markedly increases the expression of the proinflammatory cytokine TNF-α and the adhesion molecule ICAM-1 as well as the number of infiltrating neutrophils in the gastric mucosa, which play crucial roles in the progression of gastric injury (18). Activated neutrophils release inflammatory mediators capable of damaging endothelial cells, and inhibition of neutrophil infiltration prevents the stress-induced reduction of mucosal blood flow and the production of gastric lesions (27). In addition to its vasoconstrictor properties, ANG II promotes tissue inflammation, enhancing neutrophil infiltration through AT1 receptor stimulation (39, 47), with increased expression of TNF-α (23, 30, 39), ICAM-1 (45), and P-selectin (1, 35). There is a close interaction and feedback regulatory control between ANG II and TNF-α. TNF-α was reported to downregulate AT1 receptors (7, 41), which is probably a compensatory, feedback mechanism. In other models, TNF-α in combination with other proinflammatory cytokines upregulated AT1 receptors and enhanced ANG II profibrotic effects (9, 34). We present evidence that treatment with the AT1 receptor antagonist, in parallel to the protection against gastric injury, exerts anti-inflammatory actions, dramatically decreasing the stress-induced TNF-α and ICAM-1 overexpression and the neutrophil infiltration in the gastric mucosa. We report increased ICAM-1 expression in the endothelium of arteries of the gastric mucosa and submucosa, and in venules of the submucosa, where AT1 receptors are located. These findings indicate that the anti-inflammatory effects of AT1 blockade could be relevant for the protection of stress-induced gastric ulcers.

Sixth, we confirm that the gastric content of PGE2 is substantially decreased after cold restraint (6). We could interpret this finding as the result of decreased PGE2 synthesis, which increased TNF-α production by inflammatory cells, as is the case after Helicobacter pylori stimulation (46), accelerating gastric injury (3). On the other hand, ANG II increases PGE2 synthesis and release through phospholipase A2 stimulation (37), balancing its vasoconstrictor effects (42). The role of ANG II receptor types on PGE2 formation and release is controversial and may be mediated through AT2 (5, 11) or AT1 (14) receptor stimulation. ANG II-mediated increase in PGE2 synthesis and release is protective, since inhibition of prostaglandin synthesis results in ANG II stimulation of TNF-α production (30). In addition, ANG II stimulates cyclooxygenase-2 (COX-2), leading not only to inflammation but also to increased PGE2 synthesis, and this effect is mediated by AT1 receptor activation (20). The combined effects of stress and ANG II stimulation on gastric PGE2 levels may be the result of the combination of these opposing factors. Gastric PGE2 levels are also inhibited after AT1 receptor inhibition in nonstressed rats, and the decrease in prostaglandin content after treatment with the AT1 blocker was even more pronounced in rats submitted to cold restraint. This may be interpreted as the result of decreased PGE2 synthesis following inhibition of COX-2 with increased prostaglandin release by unopposed AT2 activation. However, although we found AT2 receptors in all layers of the stomach, their expression was low, and we could not detect any AT2 receptor binding after stress or AT1 blockade in the gastric mucosa. For this reason the role of AT2 receptors in cold restraint remains an open question.

The mechanisms discussed here are certainly interrelated. We believe that AT1 receptor stimulation by ANG II during stress is a major factor in the production of gastric injury. Under these conditions, feedback regulatory mechanisms that could include stimulation of synthesis and release of the injury-protective PGE2 are not sufficient to prevent stress- and ANG II-in-
duced gastric damage unless AT₁ receptors are blocked. AT₁ receptor blockade, which preserves the PGE₂ and glucocorticoid protective actions, blocks the inflammatory cascade of TNF-α, ICAM-1, and neutrophil infiltration and protects gastric blood flow by partially inhibiting the sympathoadrenal discharge and ANG II-mediated vasoconstriction. By eliminating the vasoconstrictor, proinflammatory effects of ANG II and preserving the protective actions, AT₁ antagonists almost completely prevent stress-induced ulcer formation.

Ours is the first demonstration that inhibition of ANG II AT₁ receptors, by combined local and systemic mechanisms, protects gastric blood flow, inhibits the proinflammatory cascade, and preserves the protective mechanisms including the natural pituitary-adrenal response to stress, preventing the gastric ischemia and inflammation characteristic of a major stress response and protecting the gastric mucosa from stress-induced ulcerations. Our observations may represent a paradigm for novel, anti-inflammatory actions by this class of drugs. It is reasonable to consider the possibility of a beneficial protective and therapeutic effect of AT₁ antagonists against acute stress-induced gastric injury.

Alterations in gastric blood flow and increased inflammation play significant roles in other models of stress-induced gastric injury (26) and in the production of gastric lesions following infection with H. pylori (46) and nonsteroidal anti-inflammatory drug use (52). The possible role of ANG II in these models, however, has not been explored. If ANG II is important in other models of gastric injury, the use of AT₁ antagonists could be proposed to prevent the development and recurrence of gastric lesions in a variety of clinical conditions.

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