ET$_A$ and ET$_B$ receptor function in pancreatitis-associated microcirculatory failure, inflammation, and parenchymal injury

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INAPPROPRIATE EXTRALUMINAL activation of digestive enzymes followed by autodigestion of the gland is still predominantly considered the key mechanism in the pathogenesis of severe acute bile-induced pancreatitis (5, 26, 34, 46). However, there is increasing evidence that microvascular alterations may critically contribute to the development of pancreatitis-associated acinar cell necrosis (7, 12, 24, 25, 27, 29, 34). These microvascular alterations may include endothelial cell activation with release of endothelins (ETs) (2, 3, 49), which are well known as the most potent long-acting vasoconstrictors (49).

ETs exist in three different isoforms (ET-1, ET-2, ET-3) (22), which are found not only in endothelial cells but also in other cell types of nervous, respiratory, urinary, and gastrointestinal tissue (17, 18, 21, 47). Expression of ETs and the corresponding receptors have also been demonstrated for the exocrine pancreas (19). Moreover, in the pancreatic gland, ETs have been shown to cause a deterioration of nutritive blood flow, an inflammatory response, and acinar cell necrosis (38, 39), which reflect the three distinct characteristics of acute necrotizing pancreatitis.

In acute experimental pancreatitis, both improvement and aggravation of disease have been reported as a result of ET-1 application (28, 31). The administration of a selective ET-1 receptor antagonist was shown to reduce pancreatitis-associated mortality (15), whereas simultaneous inhibition of the ETA and the ETB receptor was not capable of improving survival (13). This clearly demonstrates that the role of ETs in the pathogenesis of acute pancreatitis is still controversial and that the individual function of ETA and ETB receptors is not yet fully understood. In particular, little is known regarding the contribution of the individual ET receptor functions on the development of pancreatitis-associated microvascular injury.

Using a rat model of sodium-taurocholate-induced pancreatitis and intravital microscopy, we therefore studied whether selective inhibition of ETA receptor function or combined ETA and ETB receptor blockade affects the development of microcirculatory failure, inflammation, and parenchymal injury.

MATERIALS AND METHODS

Preparative surgery and animal model. Experiments were conducted in accordance with the German legislation on protection of animals and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council). Anesthesia and preparative surgery have been described previously in detail (40). In brief, male inbred Lewis rats (220–270 g) were anesthetized with pentobarbital sodium (50 mg/kg body wt) almost abolished pancreatitis-associated capillary recruitment and tissue injury. Furthermore, pretreatment with a selective ETA receptor antagonist (4 mg/kg body wt), which predominantly inhibits the ETA receptor, revealed an improvement of some microcirculatory disorders and a significant attenuation of leukocyte recruitment and tissue injury. Furthermore, pretreatment with a selective ETA receptor antagonist (1 µg/kg body wt) almost abolished pancreatitis-associated capillary constriction, restored functional capillary density, and, consequently, improved overall nutritive perfusion. Importantly, the maintenance of an appropriate microcirculation by selective ETA receptor inhibition was accompanied by a significant attenuation of the inflammation-associated leukocytic response and by a marked reduction of parenchymal injury. Thus our study indicates that pancreatitis-associated development of microcirculatory failure, inflammation, and parenchymal injury is caused by ETs coupling onto the ETA receptor, which therefore may represent a promising target for novel strategies in the treatment of pancreatitis.
mg/kg ip) followed by tracheotomy to facilitate spontaneous breathing. The right carotid artery was cannulated for on-line monitoring of mean arterial blood pressure and heart rate. The right jugular vein was cannulated for application of FITC-labeled erythrocytes, dyes, and fluid replacement. Simultaneously, a transverse laparotomy was performed, followed by careful mobilization of the spleen and the pancreatic gland, which allowed a gentle exteriorization of the pancreatic corpus and tail. The exteriorized gland was covered with an oxygen-impermeable foil to avoid drying of the tissue and to prevent interference with the environment. Body temperature was kept constant at 37.0 ± 0.4°C by using a warm lighting device prior to surgical preparation. During the experiments, electrolyte and fluid loss caused by laparotomy was replaced intravenously by Ringer solution (10 ml/kg body wt).

The concentration of 4 mg/kg body wt predominantly inhibits the ETA receptor. The concentration of 10 mg/kg body wt is thought to mediate a substantial increase of microvascular permeability. The hypertensive response was studied after the experiment, as well as by histological examination. For histology of pancreatic corpus and tail, formalin-fixed samples were embedded in paraffin and cut in 4-μm sections. Slides were stained with hematoxylin and eosin. Severity of acute pancreatitis was microscopically assessed by a blinded investigator according to Spärrmann et al. (45) [edema: mild = 1, moderate = 2, severe = 3; fat necrosis: <2 sections = 3, 3–5 sections = 5, >5 sections = 7; parenchymal necrosis: focal (<5%) = 3, and/or sublobular = 5, and/or lobular (>20%) = 7; hemorrhages: mild = 3, moderate = 5, severe = 7]. The onset of an inflammatory response was examined by counting chloracetate esterase (CAE)-stained leukocytes in 50 highpower fields per animal (33).

Functional capillary density (FCD) was defined as the capillary length of all RBC-perfused capillaries per observation area. According to the method of Schmidt-Schönbein et al. (44), a grid system with a grid width of 50 μm was superimposed on the monitor, and the number of intersections (N) between the grid and the capillaries was counted. The FCD was calculated according to the equation Lc = π/2·N·L, where L represents the total length of the grid system. Overall changes in total nutritive perfusion were assessed from the mean perfused capillaries intersecting with the grid system and the individual capillary volumetric blood flow. This perfusion index (PI) was calculated according to the equation PI = N/Δ·Q (pl·s⁻¹·0.01 mm⁻²), where Q represents the surface unit of the grid system, N reflects the volumetric blood flow (Q = V·π·r²), V is mean velocity; and r is radius (26). All off-line measurements were performed by a blinded evaluator.

Statistical analysis. All results are expressed as means ± SE. Statistical differences within each group were determined by repeated-measures ANOVA, which was, if significant, followed by the Student-Newman-Keuls test. Different groups were statistically compared by one-way ANOVA followed by post hoc Student-Newman-Keuls test. When criteria for parametric tests were violated, the respective non-parametric test (Friedman repeated-measures ANOVA on ranks, Kruskal-Wallis analysis, and a signed-rank test) was used. A value of P < 0.05 was considered significant.

RESULTS

Monitoring of pancreatic baseline microcirculation revealed a homogeneous perfusion pattern without re-
### Table 1. Effects of sham operation and retrograde intraductal infusion of physiological sodium solution on microcirculatory parameters

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Capillary Diameter, μm</th>
<th>RBC Velocity, μm/s</th>
<th>FCD, cm⁻¹</th>
<th>PI, pl·s⁻¹·0.01 mm⁻²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bl</td>
<td>4.6 ± 0.2</td>
<td>702 ± 107</td>
<td>531 ± 7</td>
<td>144 ± 29</td>
</tr>
<tr>
<td>10</td>
<td>4.7 ± 0.2</td>
<td>672 ± 43</td>
<td>532 ± 9</td>
<td>166 ± 17</td>
</tr>
<tr>
<td>30</td>
<td>4.4 ± 0.1</td>
<td>633 ± 22</td>
<td>533 ± 8</td>
<td>162 ± 5</td>
</tr>
<tr>
<td>60</td>
<td>4.2 ± 0.2</td>
<td>678 ± 76</td>
<td>530 ± 8</td>
<td>175 ± 26</td>
</tr>
<tr>
<td>120</td>
<td>4.3 ± 0.2</td>
<td>680 ± 37</td>
<td>533 ± 7</td>
<td>168 ± 11</td>
</tr>
<tr>
<td>Sham 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bl</td>
<td>4.3 ± 0.3</td>
<td>839 ± 85</td>
<td>539 ± 9</td>
<td>180 ± 19</td>
</tr>
<tr>
<td>10</td>
<td>4.7 ± 0.1</td>
<td>657 ± 36</td>
<td>536 ± 9</td>
<td>175 ± 16</td>
</tr>
<tr>
<td>30</td>
<td>4.3 ± 0.2</td>
<td>860 ± 49</td>
<td>531 ± 10</td>
<td>205 ± 19</td>
</tr>
<tr>
<td>60</td>
<td>4.4 ± 0.2</td>
<td>766 ± 30</td>
<td>532 ± 8</td>
<td>197 ± 22</td>
</tr>
<tr>
<td>120</td>
<td>4.1 ± 0.2</td>
<td>794 ± 82</td>
<td>538 ± 10</td>
<td>173 ± 32</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 4 rats per group. Sham 1, sham operation only; Sham 2, retrograde, volume (0.6 ml), time (4–5 min), and pressure (<35 mm Hg)-controlled intraductal infusion of physiological sodium solution into the main pancreatic duct; RBC, red blood cell; FCD, functional capillary density, PI, perfusion index, bl, baseline.

### Table 2. Mean arterial pressure and heart rate

<table>
<thead>
<tr>
<th>Time, min</th>
<th>AP, mmHg</th>
<th>AP + anti-ET&lt;sub&gt;A&lt;/sub&gt;, 4 mg/kg body wt</th>
<th>AP + anti-ET&lt;sub&gt;A&lt;/sub&gt;, 10 mg/kg body wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Arterial Pressure, mmHg</td>
<td></td>
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<td></td>
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<tr>
<td>0</td>
<td>103 ± 5</td>
<td>103 ± 8</td>
<td>103 ± 8</td>
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<tr>
<td>2</td>
<td>101 ± 5</td>
<td>105 ± 7</td>
<td>99 ± 9</td>
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<tr>
<td>6</td>
<td>99 ± 5</td>
<td>101 ± 8</td>
<td>103 ± 8</td>
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<td>96 ± 7</td>
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<td>95 ± 9</td>
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<td>96 ± 9</td>
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</tr>
<tr>
<td>120</td>
<td>101 ± 8</td>
<td>96 ± 7</td>
<td>91 ± 6</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Heart Rate, beats/min</th>
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<tbody>
<tr>
<td>0</td>
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<tr>
<td>2</td>
</tr>
<tr>
<td>6</td>
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<tr>
<td>10</td>
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<tr>
<td>20</td>
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<tr>
<td>60</td>
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<tr>
<td>90</td>
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<tr>
<td>120</td>
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</table>

Values are means ± SE; n = 8 rats per group. AP, acute pancreatitis without treatment; ET, endothelin.
induction of acute pancreatitis. However, pretreatment with the ET\textsubscript{A-B} receptor antagonist in a concentration of only 4 mg/kg body wt inhibited the pancreatitis-induced decrease in capillary diameters over the entire observation period. Selective inhibition of the ET\textsubscript{A} receptor was not only effective in also completely abolishing the pancreatitis-induced decrease in capillary diameters but it even induced a slight increase in diameters when compared with baseline conditions (Fig. 1).

**RBC velocity.** Initiation of acute pancreatitis resulted in a steep drop of RBC velocity from 654 ± 24 to 327 ± 26 μm/s, which reached its maximum at 6 min after the end of retrograde intraductal infusion of sodium taurocholate (Fig. 2). This initial decrease in RBC velocity of 47% was followed by a sustained reduction of 32–38%, reflecting a significantly decreased mean RBC velocity of 367–425 μm/s during the entire 2-h observation period. The decrease in mean RBC velocity was a consequence of an increased number of capillaries with either stasis or low-flow conditions and of a reduced number of capillaries that presented with very high flow velocity, reflecting a substantial increase in heterogeneity of RBC velocities.

Pretreatment with the combined ET\textsubscript{A} and ET\textsubscript{B} receptor antagonist in a concentration of 10 mg/kg body wt showed a transient recovery of RBC velocity after the initial decrease, whereas this receptor antagonist given in the lower concentration of 4 mg/kg body wt caused a generally less pronounced decrease in RBC velocity (Fig. 2). Compared with the capillary RBC velocity distribution after pancreatitis without treatment, both concentrations of the combined ET\textsubscript{A-B} receptor antagonist increased the number of capillaries with stasis of RBC flow, and, after application of the lower concentration, a higher number of capillaries with normal and high RBC velocities was also observed.

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**Fig. 1.** Changes in pancreatic capillary diameters after induction of experimental pancreatitis and pretreatment with either a combined endothelin (ET\textsubscript{A-B}) receptor antagonist in 2 different concentrations (4 mg and 10 mg/kg body wt) or a selective ET\textsubscript{A} receptor antagonist. Animals with acute pancreatitis without treatment (AP) served as controls. Values are means ± SE. \( P < 0.05; \) a, vs. baseline; c, vs. AP; d, vs. AP + anti-ET\textsubscript{A-B} (10 mg/kg body wt); e, vs. AP + anti-ET\textsubscript{A}.

**Fig. 2.** Changes in pancreatic red blood cell (RBC) velocities after induction of experimental pancreatitis and pretreatment with either a combined ET\textsubscript{A-B} receptor antagonist in 2 different concentrations (4 and 10 mg/kg body wt) or a selective ET\textsubscript{A} receptor antagonist. AP animals served as controls. Values are means ± SE. \( P < 0.05; \) a, vs. baseline.
Pretreatment with the selective ETA receptor antagonist caused a nonsignificant initial decrease in RBC velocity and a recovery to almost baseline values (Fig. 2). Thus, compared with pancreatitis without treatment, the selective blockade of the ETA receptor showed a less pronounced decrease of mean RBC velocity on induction of pancreatitis, which was due to only a small number of capillaries with stasis and a high number of capillaries with normal or high RBC velocities.

**FCD.** After induction of acute pancreatitis, quantitative analysis of RBC-perfused pancreatic capillaries revealed that the decline of blood flow velocity was paralleled by significant perfusion failure in ~60% of the initially perfused nutritive capillaries (Fig. 3). This was indicated by a reduction of FCD from 530 ± 12 cm⁻¹ at baseline to 183 ± 11 cm⁻¹ at 2 h after induction of pancreatitis.

Pretreatment with the combined ETA-B receptor antagonist, regardless of the concentration used, had no significant effect on the pancreatitis-related decrease of FCD when compared with pancreatitis without treatment. In contrast, pretreatment with the high concentration (10 mg/kg body wt) of the combined ETA-B receptor antagonist resulted even in a more pronounced decrease of FCD with values of 114 ± 28 cm⁻¹ at 2 h after induction of pancreatitis.

Pretreatment with the selective ETA receptor antagonist, however, caused a significant attenuation of the pancreatitis-related decrease in FCD. At 2 h after induction of pancreatitis, only ~40% of the initially perfused nutritive capillaries were found nonperfused, as was also reflected by a FCD of still 334 ± 8 cm⁻¹ (Fig. 3).

**PI.** Experimental pancreatitis was characterized by a decline of the PI to only 18–25% of the initial nutritive perfusion at baseline conditions. Simultaneous blockade of the ETA and ETB receptor (ETA-B receptor antagonist, 10 and 4 mg/kg body wt) showed an overall nutritive perfusion failure, which was slightly less pronounced but not significantly different when compared with the PI measured in animals undergoing acute pancreatitis without treatment (Fig. 4).

In contrast, selective blockade of the ETA receptor was effective in significantly attenuating the pancreatitis-related decrease in overall nutritive perfusion (Fig. 4). After pretreatment with the selective ETA receptor antagonist, a decrease of the PI to only 60–70% was calculated, which indicates improved overall nutritive perfusion, when compared with all other groups studied.

**Pancreas histology.** Histomorphology after sodium taurocholate-induced acute pancreatitis was analyzed semiquantitatively by using the Spormann score. Pancreatitis without treatment was characterized by severe interstitial edema formation, considerable necrosis of fatty tissue, distinct sublobular and lobular parenchymal necrosis, as well as hemorrhage, resulting in a Spormann score of 17 ± 1, which reflects severe, acute, necrotizing pancreatitis.

Pretreatment with the combined ETA-B receptor antagonist in a concentration of 10 mg/kg body wt had no beneficial effect on the pancreatitis-associated tissue injury, whereas the lower concentration of 4 mg/kg body wt caused a significant reduction of the tissue damage (Fig. 5). The Spormann score had improved to 11 ± 1, which was mainly due to a decrease in degree and amount of fatty tissue and parenchymal cell necrosis.

Pretreatment with the selective ETA receptor antagonist caused a further attenuation of tissue damage, which was reflected by a Spormann score of only 9 ± 1. This reduction was caused by a significant decrease in interstitial edema formation as well as by an attenuation of fatty tissue and parenchymal cell necrosis (Fig. 5).

**Inflammatory response.** The inflammatory response in acute pancreatitis is characterized by accumulation of leukocytes in postcapillary venules and infiltration of these cells into tissue. In nontreated pancreatitis, a
total of 207 ± 29 CAE-stained leukocytes were counted in 50 high-power fields, 113 ± 22 CAE-stained leukocytes per 50 high-power fields were found in the pancreatic tissue, whereas 94 ± 9 CAE-stained leukocytes per 50 high-power fields were counted in postcapillary venules (Fig. 6).

Pretreatment with the combined ETA + ETB receptor antagonist in a dose of 10 mg/kg body wt caused an increase of the number of CAE-stained leukocytes (total: 340 ± 90, tissue: 188 ± 61, postcapillary venules: 151 ± 31 per 50 high-power fields), whereas pretreatment with a dose of 4 mg/kg body wt resulted in a significant attenuation of CAE-stained leukocyte recruitment (total: 175 ± 33, tissue: 93 ± 22, postcapillary venules: 83 ± 16 per 50 high-power fields) (Fig. 6).

After pretreatment with the selective ETA receptor antagonist, only a total of 121 ± 16 CAE-stained leukocytes were counted in 50 high-power fields, which reflects a significant reduction of pancreatitis-related recruitment of activated leukocytes, when compared with nontreated pancreatitis controls (Fig. 6). More interestingly, pretreatment with the selective ETA receptor antagonist predominantly caused a decreased number of CAE-stained leukocytes (41 ± 8 per 50 high-power fields) that had already emigrated in the pancreatic tissue, whereas the number of CAE-stained leukocytes accumulating in postcapillary venules (80 ±

Fig. 4. Changes in total nutritive pancreatic perfusion (perfusion index) after induction of experimental pancreatitis and pretreatment with either a combined ETA + ETB receptor antagonist in 2 different concentrations (4 and 10 mg/kg body wt) or a selective ETA receptor antagonist. AP animals served as controls. Values are means ± SE. *P < 0.05: a, vs. baseline; b, vs. value at 6 min; c, vs. AP; d, vs. AP + anti-ETA.

Fig. 5. Pancreatic exocrine tissue damage according to the Spormann score after induction of experimental pancreatitis and pretreatment with either a combined ETA + ETB Receptor antagonist in 2 different concentrations (4 and 10 mg/kg body wt) or a selective ETA receptor antagonist. AP animals served as controls. Values are means ± SE. *P < 0.05: a, vs. AP; b, vs. AP + anti-ETA; c, vs. AP + anti-ET(A + B) (4 mg/kg body wt).

Fig. 6. Pancreatic leukocyte recruitment, as determined by counting of chloracetate esterase (CAE)-stained leukocytes in 50 high-power fields (HPF) per animal, after induction of experimental pancreatitis and pretreatment with either a combined ETA + ETB receptor antagonist in 2 different concentrations (4 and 10 mg/kg body wt) or a selective ETA receptor antagonist. AP animals served as controls. Open bars demonstrate accumulation of CAE-stained leukocytes in postcapillary venules, and solid bars represent those CAE-stained leukocytes that already emigrated into the pancreatic tissue. Values are means ± SE. *P < 0.05: b, vs. AP + anti-ETA; d, vs. AP + anti-ET(A + B) (10 mg/kg body wt).
DISCUSSION

Reflux of bile followed by an increase in the permeability of the pancreatic duct are believed to initiate acute biliary pancreatitis (37, 41), whereas autoactivation of premature pancreatic digestive enzymes is considered mainly for the progression from interstitial edema to often fatal hemorrhagic necrosis (5, 26, 34, 46). However, because experimental data that recommend free fatty acids as a promoter of acinar cell necrosis have been disappointing (20), microvascular dysfunction has been suggested as the critical pathogenic mechanism in the development and progression of the disease (7, 12, 24, 25, 27, 29). Ischemic acinar cell necrosis may be the central event in the pathogenesis of acute pancreatitis, promoting the action of toxic products to induce autodigestion (6). Accordingly, our laboratory found in a previous study (40) that the capillary network might act as the primary target of the noxious action of bile salts and that these bile salt-induced microcirculatory deteriorations are a determining factor for the intrapancreatic expansion of acinar cell necrosis. In addition, our laboratory found evidence that in normal pancreas, ETs mediate three distinct pathological events that are also characteristic for acute necrotizing pancreatitis, i.e., microcirculatory failure, inflammation, and acinar cell necrosis (38, 39). Consequently, we hypothesized that inhibition of ET receptors may reduce microcirculatory dysfunction, inflammation, and acinar cell necrosis in acute experimental pancreatitis.

Before induction of experimental pancreatitis, the animals were pretreated with either a combined ETA/B receptor antagonist or a highly selective ETA receptor antagonist. The ETA/B receptor antagonist was applied in two different concentrations, where the high concentration of 10 mg/kg body wt is thought to mediate a simultaneous inhibition of both receptors, whereas the low concentration of 4 mg/kg body wt predominantly inhibits the ETA receptor. The control group, which did not undergo acute experimental pancreatitis, proves that the experimental model was stable over time and that no deterioration was caused by the preparation. Retrograde intraductal infusion of physiological sodium solution caused no significant deterioration of microcirculatory or histological parameters, which excludes nonspecific pressure effects as the cause of experimental pancreatitis (40). The difference in mean baseline capillary diameters between the untreated control groups and the groups pretreated reflects inhibition of ET caused vasoconstriction by ET receptor antagonists.

Pretreatment with the high concentration of the ETA/B receptor antagonist showed no significant improvement of pancreatitis-induced microcirculatory injury when compared with animals that underwent pancreatitis without treatment. Evaluation of the magnitude of perfusion failure by determination of the FCD revealed an even higher number of nonperfused capillaries after pretreatment. Accordingly, evaluation of tissue damage and inflammatory response caused by experimental pancreatitis proved an aggravation rather than an improvement as a result of pretreatment with the high dose of the ETA/B receptor antagonist.

In contrast, pretreatment with the low concentration of the ETA/B receptor antagonist revealed a significantly less pronounced reduction of capillary diameters, whereas deterioration of RBC velocity, FCD, and PI were not affected. However, determination of leukocyte recruitment and parenchymal tissue injury still displayed a significant attenuation when compared with nontreated pancreatitis controls. Furthermore, the selective inhibition of the ETA receptor was effective to almost abrogate the pancreatitis-induced microcirculatory disorders, parenchymal injury, and inflammatory response. The decline in overall leukocyte recruitment was caused by preventing leukocytes to emigrate into the pancreatic tissue, whereas leukocyte accumulation in postcapillary venules remained almost unchanged, compared with the nontreated pancreatitis group.

This might suggest that selective inhibition of the ETA receptor prevents migration of leukocytes in acute experimental pancreatitis, which is in accordance with findings demonstrating that ET-1 ETA receptor interaction plays a key role in leukocyte adhesion and migration (8, 10, 23, 43, 51). Furthermore, there is an increasing body of evidence that ETA receptor antagonists inhibit ET-1-mediated adhesion and migration of leukocytes, whereas selective ETB receptor antagonists are ineffective (10, 43, 51).

The fact that simultaneous inhibition of ETA and ETB receptors caused no improvement, whereas more pronounced or selective inhibition of the ETA receptor did improve the time course of experimental pancreatitis, might be explained by the physiological function of the ETB receptor. Stimulation of the ETB receptor mediates the clearance of ETs and the liberation of nitric oxide, which counteracts the ET-mediated vasoconstriction (16, 32, 36, 42). Therefore, simultaneous inhibition of ETA and ETB receptors results in both sustained high local ET concentrations and deficiency of nitric oxide-mediated vasodilation, which may mask the beneficial effect of inhibition of the ETA receptor. This might also explain that simultaneous, maximal inhibition of ETA and ETB receptors caused some aggravation of microcirculatory disorders, and it extends the knowledge based on results of others who could not demonstrate a survival benefit in acute experimental pancreatitis after treatment with the combined ETA/B receptor antagonist bosentan (13). This is also in compliance with the current opinion that selective ETA receptor antagonists have a number of still hypothetical advantages over nonselective ET receptor antagonists (1). Moreover, the role of the ETB receptor in circulatory homeostasis is known to be ambiguous as

8 per 50 high-power fields) remained almost unchanged, compared with the nontreated pancreatitis group.
reflected in either vasodilation or vasoconstriction ascribed to the receptor, whereas the clearing function for ET-1 of the ETB receptor seems to be of pivotal importance (42). In addition, a more recent study (11) could not demonstrate significant benefits for selective ETB receptor inhibition in acute experimental pancreatitis.

Our results showing that selective inhibition of the ETA receptor attenuates pancreatitis-induced microcirculatory failure, inflammation, and parenchymal injury is in good concordance with the finding that transgenic rats with ET-1 receptor overexpression develop a more severe pancreatitis and that the administration of a selective ET-1 receptor antagonist in acute pancreatitis reduces fluid sequestration, improves microcirculation, and ameliorates mortality (14, 15).

Overall, these findings implicate the pivotal contribution of the ETA receptor in the pathogenesis of the pancreatitis-associated microcirculatory failure, and they underline the key function of ETs in acute necrotizing pancreatitis.

In normal pancreas, ETs and especially ET-1 mediate microcirculatory deteriorations, which are associated with local inflammation and intrapancreatic tissue damage (38, 39). These recent studies have also shown that the magnitude of microcirculatory failure is strictly related to the amount of final tissue damage, indicating that ischemia may act as a major cause of acinar cell necrosis. In accordance, it has been shown that temporary short-term pancreatic ischemia causes reversible functional and morphological changes (4, 25), whereas long-lasting ischemia results in necrotizing pancreatitis (30) and that therapeutic approaches, which aim at preventing microcirculatory stasis, limit the extent of pancreatic necrosis (24). In line with this, the present study now demonstrates that selective inhibition of the ETA receptor causes a significant attenuation of the pancreatitis-associated nutritive perfusion failure, which is paralleled by a significant reduction of pancreatitis-associated inflammation and tissue injury. Thus we conclude that microcirculatory failure with subsequent ischemia caused by ETs coupling onto the ETA receptor is a determining factor in the pathogenesis of acute necrotizing pancreatitis and that inhibition of the ETA receptor function may represent an interesting, novel treatment approach.

REFERENCES