NO and prostanoids blunt endothelin-mediated coronary vasoconstrictor influence in exercising swine

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Merkus, Daphne, Oana Sorop, Birgit Houweling, Frans Boomsma, Anton H. van den Meiracker, and Dirk J. Duncker. NO and prostanoids blunt endothelin-mediated coronary vasoconstrictor influence in exercising swine. Am J Physiol Heart Circ Physiol 291: H2075–H2081, 2006. First published June 2, 2006; doi:10.1152/ajpheart.01109.2005.—Withdrawal of the endothelin (ET)-mediated vasoconstrictor influence contributes to metabolic coronary vasodilation during exercise. Because production of nitric oxide (NO) and prostanoids increases with increasing shear stress and because NO and prostanoids are able to modify the release of ET, we hypothesized that the withdrawal of ET-mediated coronary vasoconstriction during exercise is mediated through NO and/or prostanoids. To test this hypothesis, 19 chronically instrumented swine were studied at rest and while running on a treadmill up to 85–90% of maximal heart rate. Blockade of ET\textsubscript{A}/ET\textsubscript{B} receptors with tezosentan resulted in an increase in coronary venous O\textsubscript{2} consumption (MV\textsubscript{O}2) to maintain a consistently high level of myocardial O\textsubscript{2} extraction (MEO\textsubscript{2}) (9, 15, 33). This tight coupling has been proposed to depend on vasodilator signals released from cardiomyocytes, such as adenosine, and/or the endothelium, such as nitric oxide (NO) and prostacyclin (PGI\textsubscript{2}) (6, 15). Indeed, the intrinsic state of coronary resistance vessels is one of marked vasoconstriction, with messengers being generated by cardiomyocytes and endothelium to cause vasorelaxation (11, 20, 34). In addition, it has been proposed that the coronary vasodilation that occurs during exercise principally depends on the increased influence of vasodilator mechanisms (11). Interestingly, Merkus et al. (20, 21) recently obtained evidence to support the concept that in addition to the increased influence of vasodilator mechanisms, the withdrawal of vasoconstrictor influences [i.e., endothelin (ET)] may also contribute to the exercise-induced coronary vasodilation. Thus, under basal resting conditions with low myocardial metabolism, ET contributes to maintenance of vascular tone, whereas during increased metabolic demand associated with treadmill exercise, the vasoconstrictor influence of ET is attenuated, thereby contributing to metabolic vasodilation in the coronary vasculature.

Under physiological conditions, a delicate balance exists between vasodilator (NO and PGI\textsubscript{2}) and vasoconstrictor (ET) factors produced by the endothelium that contributes to the optimization of vascular tone control. Thus both NO and PGI\textsubscript{2} can limit the production and/or release of ET (12, 28). Moreover, binding of ET to the ET\textsubscript{B} receptor on the endothelium results in the release of NO and PGI\textsubscript{2} (30). Because NO and prostanoid production are stimulated by an increase in shear stress (14, 26), as occurs during exercise, these factors may contribute to metabolic vasodilation not only directly but also indirectly through inhibition of ET-mediated vasoconstriction. Such interaction is beneficial because ET is one of the most potent vasoconstrictors known and its overproduction is thought to play a role in the etiology of cardiovascular disease states, such as systemic and pulmonary hypertension, which are also characterized by endothelial dysfunction and decreased NO bioavailability.

Consequently, the aim of the present study was to investigate whether the waning of ET-mediated vasoconstriction in the coronary circulation with incremental levels of exercise is mediated through NO and/or prostanoids. For this purpose, the effect of ET receptor blockade with tezosentan was studied in exercising swine, in the absence and presence of single or combined blockade of NO and prostanoid synthesis.

METHODS

Animals

Studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996), and with prior approval of the Animal Care Committee of the Erasmus Medical Center. Nineteen 2- to 3-mo-old Yorkshire × Landrace pigs (21.6 ± 0.5 kg at the time of surgery) of either sex (7 males and 12 females) were entered into the study.
Surgery

Pigs were sedated with ketamine (20 mg/kg im), anesthetized with thiopental sodium (10–15 mg/kg iv), intubated, and ventilated with a mixture of O₂ and N₂O (1:2) to which 0.2–1% (vol/vol) isoflurane was added (8, 21). Anesthesia was maintained with midazolam (2 mg/kg + 1 mg·kg⁻¹·h⁻¹·iv) and fentanyl (10 μg·kg⁻¹·h⁻¹·iv). Under sterile conditions, the chest was opened via the fourth left intercostal space, and a fluid-filled polyvinylchloride catheter was inserted into the aortic arch for aortic blood pressure measurement (Combitrans pressure transducers, Braun) and blood sampling. A microtipped pressure transducer (P₂₅, Konigsberg Instruments) was inserted into the left ventricle via the apex. Polyvinylchloride catheters were inserted into the left atrium to measure pressure, and into the pulmonary artery to administer drugs. A small angiocatheter was inserted into the left anterior descending coronary venous blood sampling. Finally, a transit-time flow probe (Transonic Systems) was placed around the left anterior descending coronary artery (21). Catheters were tunneled to the back, and animals were allowed to recover, receiving analgesia (0.3 mg buprenorphine im) for 2 days and antibiotic prophylaxis (25 mg/kg amoxicillin and 5 mg/kg gentamicin iv) for 5 days (8, 21).

Experimental Protocols

Studies were performed 1–3 wk after surgery with animals resting and exercising on a motor-driven treadmill up to 85–90% of maximal heart rate. Four exercise protocols were performed on different days and in random order. Unless otherwise mentioned, all chemicals were obtained from Sigma.

ET receptor blockade. With swine (n = 15) lying quietly on the treadmill, resting hemodynamic measurements consisting of heart rate, LV pressure, first derivative of LV pressure, mean aortic pressure, left atrial pressure, and CBF were obtained and blood samples collected. Hemodynamic measurements were repeated, and rectal temperature was measured with animals standing on the treadmill. Subsequently, a five-stage (1–5 km/h) treadmill exercise protocol was started; each exercise stage lasted 3 min. Hemodynamic variables were continuously recorded and blood samples collected during the last 60 s of each stage when steady state was achieved. After the exercise protocol was completed, animals were allowed to rest on the treadmill for 90 min after which animals received the mixed ETA/ETB receptor antagonist tezosentan [3 mg/kg iv, followed by 6 mg·kg⁻¹·h⁻¹·iv (21); a gift from Dr. Clozel, Actelion Pharmaceuticals], and the exercise protocol was repeated.

NO and ET receptor blockade. Ninety minutes after swine (n = 10) had undergone a control exercise trial, animals received NO synthase inhibitor Nω-nitro-L-arginine [L-NAME, 20 mg/kg iv (7)] and underwent a second exercise trial. Ninety minutes later, tezosentan (3 mg/kg iv + 6 mg·kg⁻¹·h⁻¹·iv) was given to the animals and they underwent a third exercise trial. Because L-NAME has a long-lasting effect (mean arterial pressure, 122 ± 3 mmHg after administration of L-NAME before the second exercise protocol and 119 ± 3 mmHg before administration of tezosentan before the third exercise protocol), no additional L-NAME was administered before the third exercise protocol.

Prostanoids and ET receptor blockade. Ninety minutes after swine (n = 9) had undergone a control exercise trial, animals received the cyclooxygenase inhibitor indomethacin [10 mg/kg iv over 10 min (22)], and, 5 min later, underwent a second exercise trial. Ninety minutes later, indomethacin [5 mg/kg iv (10)] and tezosentan (3 mg/kg iv + 6 mg·kg⁻¹·h⁻¹·iv) were infused, and animals underwent a third exercise trial. The additional administration of indomethacin resulted in comparable hemodynamics (e.g., mean arterial pressure was 124 ± 7 mmHg after indomethacin before the second exercise protocol and was 124 ± 3 mmHg after indomethacin just before administration of tezosentan before the third exercise protocol).

NO, Prostanoids, and ET receptor blockade. Swine (n = 8) underwent an exercise trial in the presence of L-NAME (20 mg/kg iv). Ninety minutes after the first exercise trial, animals received indomethacin (10 mg/kg iv) and underwent a second exercise trial. Ninety minutes later, a combined blockade of NO, PGI₂, and ET with indomethacin (5 mg/kg iv) and tezosentan (3 mg/kg iv and 6 mg·kg⁻¹·h⁻¹·iv) was induced, and animals underwent a third exercise trial.

We have previously shown excellent reproducibility of the hemodynamic response in consecutive bouts of exercise (7, 8).

Blood-Gas Measurements

Blood samples were kept in iced syringes until the conclusion of each exercise trial. Measurements of P O₂ (in mmHg), P CO₂ (in mmHg), and pH were then immediately performed with a blood-gas analyzer (model 505, Acid-Base Laboratory, Radiometer, Copenhagen, Denmark). O₂ saturation (SO₂) and Hb (in grams/100 ml) were measured with a hemoximeter (OSM2, Radiometer). Myocardial O₂ delivery (MDO₂), myocardial O₂ consumption (MVO₂), and MEO₂ (MEO₂ = MVO₂/MDO₂) were computed using the blood-gas values and CBF (8).

Determination of Plasma Levels of ET

Plasma levels of ET were determined as previously described (21). In short, arterial and coronary venous blood samples (5 ml) were collected at rest (lying) and at 5 km/h in the control exercise protocol and kept on ice until the end of the exercise trial. The blood samples were then spun down, and plasma was stored at −80°C. Plasma levels of ET-like immuno-reactivity were determined by using a radioimmunoassay from Euro-Diagnostica (Malmo, Sweden), which has a cross-reactivity of 100% toward ET-1, 48% toward ET-2, and 109% toward ET-3. Because production of ET-2 and ET-3 appears to be absent in the cardiovascular system of the pig (13), the concentration measured with the radioimmunoassay most likely represents ET-1.

We found in preliminary experiments that the addition of L-NAME or indomethacin to porcine plasma resulted in a false positive readout of the RIA used to measure ET. Because it is very difficult to estimate the exact plasma concentrations of L-NAME and indomethacin, it is difficult to estimate which part of the measured increase in ET concentration after administration of these drugs is due to a true increase in plasma ET and which part is due to the presence of L-NAME and/or indomethacin in the blood sample. However, because the false positive influence of the administration of L-NAME and indomethacin is likely to be similar in arterial and coronary venous blood, it is possible to use the arteriovenous ET difference (calculated as coronary venous ET plasma concentration minus arterial ET plasma concentration) as a qualitative reflection of coronary ET production. Consequently, in a subset of animals, we obtained arterial and coronary venous plasma samples in which ET was measured.

Data Analysis

Hemodynamic data were digitally recorded and analyzed off-line. Hemodynamic variables and arteriovenous differences in plasma ET were analyzed by using ANOVA for repeated measures. Post hoc testing was done with the use of Dunnett’s test. The relationships between MVO₂ and coronary venous SO₂ (Scv O₂), coronary venous Po₂ (Pcv O₂), and MEO₂ were analyzed by using regression analysis with animal as a dummy variable. Statistical significance was accepted when P < 0.05. Data are presented as means ± SE. Because no significant differences were found between male and female swine, data from both sexes were pooled.
RESULTS

Response to Exercise

Exercise resulted in increases in heart rate (up to 80% of maximum heart rate) and left atrial pressure, whereas mean aortic blood pressure was minimally affected (Table 1). Under control conditions, the exercise-induced increase in the metabolic demand of the myocardium was accurately matched by an increase in CBF (Table 2), so that \( \text{MEO}_2 \), \( \text{ScVO}_2 \), and \( \text{PcVO}_2 \) were maintained constant (Fig. 1).

ET Receptor Blockade

Mixed ET\(_A\)/ET\(_B\) receptor blockade with tezosentan resulted in vasodilation at rest, i.e., the myocardial blood supply increased in slight excess of myocardial O\(_2\) demand, as evidenced by the decreased MEO\(_2\) and the increased ScVO\(_2\) and PcVO\(_2\). This effect of tezosentan waned with incremental levels of exercise, indicating that withdrawal of ET-mediated tone contributes to exercise-induced coronary vasodilation (Fig. 1). In contrast, the arteriovenous difference in plasma ET levels did not change during exercise (Table 3).

Interaction Among NO, Prostanoids, and ET

NO and ET receptor blockade. Inhibition of NO production with l-NNA resulted in vasoconstriction, i.e., a decrease in myocardial blood supply independent of changes in myocardial O\(_2\) demand as reflected in an increased MEO\(_2\) and a decreased ScVO\(_2\) and PcVO\(_2\) (Fig. 1). Subsequent administration of tezosentan

Table 1. Effect of ET\(_A\)/ET\(_B\) Receptor blockade on hemodynamics in the presence and absence of NO synthase inhibition and/or cyclooxygenase inhibition

<table>
<thead>
<tr>
<th></th>
<th>HR, beats/min</th>
<th>MAP, mmHg</th>
<th>LVSP, mmHg</th>
<th>LV dP/dt.max, mmHg/s</th>
<th>LAP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>130±4</td>
<td>95±3</td>
<td>107±3</td>
<td>2,720±110</td>
<td>4±1</td>
</tr>
<tr>
<td>Tezno</td>
<td>149±5†‡</td>
<td>89±3*</td>
<td>112±4</td>
<td>3,380±200*</td>
<td>2±1</td>
</tr>
<tr>
<td>l-NNA</td>
<td>103±6†</td>
<td>112±5‡</td>
<td>113±7</td>
<td>3,800±200</td>
<td>5±1</td>
</tr>
<tr>
<td>l-NNA + Tezno</td>
<td>118±5†‡</td>
<td>112±5‡</td>
<td>113±7</td>
<td>3,800±200*</td>
<td>5±1</td>
</tr>
<tr>
<td>Indo</td>
<td>86±7†</td>
<td>112±5‡</td>
<td>113±7</td>
<td>3,800±200*</td>
<td>5±1</td>
</tr>
<tr>
<td>Indo + Tezno</td>
<td>97±7†‡</td>
<td>112±5‡</td>
<td>113±7</td>
<td>3,800±200*</td>
<td>5±1</td>
</tr>
<tr>
<td>l-NNA + Indo</td>
<td>82±4‡</td>
<td>112±5†‡</td>
<td>113±7</td>
<td>3,800±200*</td>
<td>5±1</td>
</tr>
<tr>
<td>l-NNA + Indo + Tezno</td>
<td>101±8‡</td>
<td>112±5‡</td>
<td>113±7</td>
<td>3,800±200*</td>
<td>5±1</td>
</tr>
</tbody>
</table>

Values are means ± SE. ET, endothelin; NO, nitric oxide; HR, heart rate; MAP, mean aortic pressure; LVSP, left ventricular (LV) peak systolic pressure; LV dP/dt.max, maximum rate of rise of LV pressure; LAP, left atrial pressure; Tez, tezosentan; l-NNA, N\(^{\text{N}}\)-nitro-l-arginine; Indo, indomethacin. \*\( P < 0.05 \) vs. rest lying; †\( P < 0.05 \) vs. control; ‡\( P < 0.05 \), effect of Tez.
entan resulted in vasodilation at rest that was similar to the effect of tezosentan under control conditions. Moreover, the arteriovenous difference in plasma ET was not affected by l-NNA under resting conditions (Table 3), indicating that the ET-mediated vasoconstrictor influence on the coronary vasculature is not modified by NO under resting conditions. In contrast, the vasodilator effect of tezosentan did not wane during exercise after inhibition of NO production (Fig. 1), whereas the arteriovenous difference in plasma ET increased during exercise after inhibition of NO production (Fig. 1), but did not affect the arteriovenous difference in ET at rest or during exercise (Table 3). Subsequent administration of tezosentan resulted in vasodilation at rest that was similar to the vasodilation produced by tezosentan under control conditions as well as after individual blockade of NO synthase or cyclooxygenase. However, after combined blockade of NO synthase and cyclooxygenase, the vasodilation induced by tezosentan increased significantly during exercise (Fig. 1). These findings indicate that NO and prostanoids act in an additive manner to inhibit the vasoconstrictor influence of ET on the coronary resistance vessels during exercise.

**Prostanoids and ET receptor blockade.** Inhibition of cyclooxygenase with indomethacin resulted in marked coronary vasoconstriction both at rest and during exercise, as evidenced by the increased MEO$_2$ and the decreased ScV$_O_2$ and PcvO$_2$ (Fig. 1) but did not affect the arteriovenous difference in ET at rest or during exercise (Table 3). Subsequent administration of tezosentan resulted in vasodilation at rest that was similar to the vasodilation induced by tezosentan under control conditions. However, after cyclooxygenase inhibition, the vasodilator effect of tezosentan did not wane during exercise (Fig. 1), indicating that prostanoids act to blunt the coronary vasoconstrictor influence of ET during exercise.

**NO, prostanoids, and ET receptor blockade.** Blockade of both NO synthase and cyclooxygenase showed an additive effect and resulted in severe coronary vasoconstriction as indicated by MEO$_2$ of over 90% (Fig. 1). Subsequent administration of tezosentan resulted in modest vasodilation at rest that was similar to the vasodilation produced by tezosentan under control conditions as well as after individual blockade of NO synthase or cyclooxygenase. However, after combined blockade of NO synthase and cyclooxygenase, the vasodilation induced by tezosentan increased significantly during exercise (Fig. 1). These findings indicate that NO and prostanoids act in an additive manner to inhibit the vasoconstrictor influence of ET on the coronary resistance vessels during exercise.

**Discussion**

The main findings of the present study are that inhibition of NO or prostanoid production augments the vasoconstrictor influence of ET on coronary resistance vessels during exercise. Our findings imply that, besides their direct vasodilator influence, NO and prostanoids also induce vasodilation by reducing the vasoconstrictor influence of ET during exercise.

**Methodological Considerations**

**Myocardial O$_2$ balance as index of vascular tone.** Under basal resting conditions, the heart is characterized by a high level (80%) of MEO$_2$ (9, 15, 33). Consequently, the ability of the coronary resistance vessels to dilate in response to increments in myocardial O$_2$ demand is extremely important to maintain an adequate O$_2$ supply. A sensitive way to study alterations in coronary vascular tone in relation to myocardial metabolism is the relationship between coronary venous O$_2$ levels and MVO$_2$ (8, 11, 21, 33). Thus an increase in coronary resistance vessel tone will limit CBF and, hence, myocardial

### Table 2. Effect of ET$_A$/ET$_B$ receptor blockade on myocardial oxygen balance in the presence and absence of NO synthase inhibition and/or cyclooxygenase inhibition

<table>
<thead>
<tr>
<th></th>
<th>Lying</th>
<th>Exercise Level, km/h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>CBF, ml/min</td>
<td></td>
<td>56±4*</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>62±8‡</td>
</tr>
<tr>
<td>Tezo</td>
<td></td>
<td>58±6</td>
</tr>
<tr>
<td>l-NNA</td>
<td></td>
<td>68±8‡</td>
</tr>
<tr>
<td>l-NNA + Tezo</td>
<td></td>
<td>39±5</td>
</tr>
<tr>
<td>Indo</td>
<td></td>
<td>44±5‡</td>
</tr>
<tr>
<td>Indo + Tezo</td>
<td></td>
<td>49±5</td>
</tr>
<tr>
<td>l-NNA + Indo</td>
<td></td>
<td>65±10</td>
</tr>
<tr>
<td>l-NNA + Indo + Tezo</td>
<td></td>
<td>210±19</td>
</tr>
<tr>
<td>MVO$_2$, μmol/min</td>
<td></td>
<td>241±24‡</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>204±30</td>
</tr>
<tr>
<td>Tezo</td>
<td></td>
<td>247±32‡</td>
</tr>
<tr>
<td>l-NNA</td>
<td></td>
<td>169±26</td>
</tr>
<tr>
<td>Indo</td>
<td></td>
<td>194±24‡</td>
</tr>
<tr>
<td>Indo + Tezo</td>
<td></td>
<td>220±18</td>
</tr>
<tr>
<td>l-NNA + Indo</td>
<td></td>
<td>260±34</td>
</tr>
<tr>
<td>l-NNA + Indo + Tezo</td>
<td></td>
<td>97±2</td>
</tr>
<tr>
<td>P$_O_2$, art, mmHg</td>
<td></td>
<td>99±3</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>98±2</td>
</tr>
<tr>
<td>Tezo</td>
<td></td>
<td>95±3</td>
</tr>
<tr>
<td>l-NNA</td>
<td></td>
<td>115±5‡</td>
</tr>
<tr>
<td>Indo</td>
<td></td>
<td>115±4†</td>
</tr>
<tr>
<td>Indo + Tezo</td>
<td></td>
<td>125±6</td>
</tr>
<tr>
<td>l-NNA + Indo</td>
<td></td>
<td>111±8</td>
</tr>
<tr>
<td>l-NNA + Indo + Tezo</td>
<td></td>
<td>210±19</td>
</tr>
</tbody>
</table>

Values are means ± SE. CBF, coronary blood flow; MVO$_2$, myocardial O$_2$ consumption; P$_O_2$, arterial O$_2$ tension. *P < 0.05 vs. rest lying; †P < 0.05 vs. control; ‡P < 0.05, effect of Tezo.
O2 supply at a given level of MV˙O2, forcing the myocardium to increase its MEO2 (to meet myocardial O2 demand), which results in a lower coronary venous O2 level. Conversely, a decrease in resistance vessel tone increases myocardial O2 supply at a given level of MV˙O2, resulting in an increased coronary venous O2 level. The coronary venous O2 level thus represents a sensitive index of myocardial tissue oxygenation (i.e., the balance between myocardial O2 supply and O2 demand), which is determined by the coronary resistance vessel tone.

ET levels. In a previous study from our laboratory (21), we found that coronary plasma ET levels did not change during exercise and were 100-fold too low to actually cause vasodilatation. This apparent contradiction with the observed vasodilator effect of tezosentan is most likely explained by the predominantly abluminal release of ET, which cannot be measured in our model. However, the ET plasma levels are thought to reflect spillover from tissue and therefore to represent to some extent what occurs in the tissue.

In the present study we found that both prostanoids and NO are involved in the regulation of coronary vasomotor tone, both at rest and during exercise. The importance of NO and prostanoids in the regulation of coronary vasomotor tone varies between species. In dogs, inhibition of cyclooxygenase has no apparent effect on coronary vasomotor tone (4), and inhibition of NO synthase resulted either in slight vasoconstriction (35) or had no effect (1, 11). In contrast, in swine and...
humans, NO and prostanoids exert a tonicvasodilator influence on the coronary vasculature (5, 7, 22, 27). In the present study, prostanoids seem to play a more important role in coronary vascular tone control than NO, because the increase in MEO₂ and the decrease in coronary venous O₂ levels are larger on administration of indomethacin than after administration of 1-NNa. Moreover, combined blockade of NO and prostanoid production shows an additive effect of those two vasodilator systems on coronary resistance vessel tone.

Similar to previous observations in our laboratory, ET receptor blockade with tezosentan resulted in vasodilation at rest under control conditions. Inhibition of NO synthase or cyclooxygenase did not influence the ET-mediated vasoconstriction influence at rest, suggesting that the vasoconstrictor influence of ET is not influenced by NO or prostanoids under resting conditions. Inhibition of both NO synthase and cyclooxygenase tended to reduce the contribution of ET to coronary vasomotor tone. Although these findings are difficult to explain, it is possible that under resting conditions, the intense vasoconstriction by NO synthase and cyclooxygenase inhibition, which resulted in a near maximal MEO₂ (92–93%) and extremely low coronary venous O₂ levels, caused insufficient blood supply and ischemia, especially in the subendocardium. Because myocardial ischemia has been shown to confer vascular protection against ET-1-induced constriction via production of adenosine and activation of A₂ receptors (23), it is possible that an increased adenosine production masked the effect of loss of NO and prostanoids on the ET-mediated vasoconstriction. Future experiments, using adenosine receptor antagonists, are needed to address this issue.

The tezosentan-induced vasodilation waned with increasing exercise intensity under control conditions, whereas the arteriovenous ET difference was not affected. Our findings that inhibition of either NO synthase or cyclooxygenase was able to uncover vasodilation by tezosentan during exercise suggest that an increased release of NO and prostanoids during exercise is responsible for a reduced contribution of ET to vascular tone control during exercise. Moreover, the observation that the effect of tezosentan increased with increasing exercise intensity when both NO synthase and cyclooxygenase were blocked suggests that these vasodilator systems can act in concert to limit the vasoconstrictor influence exerted by endogenous ET during exercise. The uncovering of ET-mediated vasoconstriction during exercise is not a generalized response to blockade of a vasodilator but is specific for NO and prostanoids because preliminary experiments (n = 3) in our laboratory indicated that the effect of tezosentan still waned during exercise after blocking adenosine receptors with 8-phenyltheophylline. Thus the tezosentan-induced increase in PcO₂ amounted 2.6 ± 1.3 mmHg at rest and was 0.2 ± 0.7 mmHg at 5 km/h, whereas the increase in ScvO₂ was 5.5 ± 2.0% at rest and only 0.7 ± 1.0% at 5 km/h.

There are several other studies that suggest interactions among the three endothelial vasoactive substances at various levels. First, ET can stimulate the production of NO and prostanoids either directly, via activation of the ETB receptor on the endothelium (3, 32), or indirectly through increased shear stress as a result of ET-induced vasoconstriction (32). These pathways provide a break for the potent ET-induced vasoconstriction. However, because plasma levels of ET are constant at rest and during exercise and because we have previously shown that the ETB receptor exerts a small tonic vasodilator effect on the coronary vasculature (21), it is not likely that ETB-induced release of NO and prostanoids contributes to a withdrawal of ET-mediated vasoconstriction during exercise. Second, both NO (2, 12) and prostanoids (29) are capable of inhibiting ET production and release via a cGMP-dependent pathway. Although inhibition of cyclooxygenase did not affect the arteriovenous ET difference, inhibition of NO synthase produced an increase in the arteriovenous ET difference during exercise, suggesting that NO limits the release of ET during exercise. Third, NO and prostanoids may also modify ET-induced vasoconstriction through altering ETA receptor sensitivity. More specifically, it has been shown that NO reduces the ETA receptor binding affinity in human vasculature (36, 37). Because our laboratory (21) has previously shown that the waning of the vasoconstrictor influence of ET with progressive exercise intensity is mediated through reduction of ETA-mediated vasoconstriction while the vasodilator influence of the ETB receptor is maintained, it is likely that the persistent vasoconstrictor effect of ET during exercise in the presence of inhibition of endothelial NO synthase or cyclooxygenase is due to sustained ETA receptor activation. To assess the contributions of each of these possible mechanisms, future experiments in exercising swine (in the absence and presence of blockade of endothelial NO synthase and cyclooxygenase) are required to measure the vasoconstriction to exogenous ET infusions and assess the responses to selective ETA and ETB receptor antagonists.

Clinical Implications

The results of the present study demonstrate that under conditions of endothelial dysfunction, characterized by a decreased production of NO and vasodilator prostanoids, the ET vasoconstrictor influence increases during exercise. These findings are clinically relevant because a growing amount of evidence suggests that endothelial dysfunction, resulting in reduced NO bioavailability, is associated with augmented ET plasma levels in pathological situations, such as coronary artery disease, myocardial infarction, and hypertension (16–19, 24), which are predictive of unfavorable clinical outcome (25, 31). Our findings indicate that loss of endothelial-derived vasodilator substances will result in enhanced coronary vasoconstriction not only through loss of their direct vasodilator influence but also via an increase in ET-mediated coronary vasoconstriction.

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REFERENCES


