Interaction between prostanoids and nitric oxide in regulation of systemic, pulmonary, and coronary vascular tone in exercising swine

Daphne Merkus, Birgit Houweling, Alisina Zarbanou, and Dirk J. Duncker. Interaction between prostanoids and nitric oxide in regulation of systemic, pulmonary, and coronary vascular tone in exercising swine. Am J Physiol Heart Circ Physiol 286: H1114–H1123, 2004. First published October 16, 2003; 10.1152/ajpheart.00477.2003.—Prostacyclin and nitric oxide (NO) are produced in the endothelium in response to physical forces such as shear stress. Consequently, both NO and prostacyclin may increase during exercise and contribute to metabolic vasodilation. Conversely, NO has been hypothesized to inhibit prostacyclin production. We therefore investigated the effect of cyclooxygenase (COX) inhibition on exercise-induced vasodilation of the porcine systemic, pulmonary, and coronary beds before and after inhibition of NO production. Swine were studied at rest and during treadmill exercise at 1–5 km/h, before and after COX inhibition with indomethacin (10 mg/kg iv), and in the absence of NO synthase inhibition with N\textsuperscript{\textbeta}-nitro-L-arginine (l-NNA; 20 mg/kg iv). COX inhibition produced systemic vasoconstriction at rest, which waned during exercise. The systemic vasoconstriction by COX inhibition was enhanced after l-NNA, which waned during exercise. Moreover, a prostanoid influence in the pulmonary circulation could not be detected after l-NNA. In contrast, COX inhibition had no effect on the pulmonary circulation, either at rest or during exercise. Consequently, the first aim of the present study was to determine the contribution of prostacyclin in the regulation of systemic, pulmonary, and coronary vascular tone in chronically instrumented swine undergoing treadmill exercise.

Several studies have suggested that an interaction exists between NO and prostaglandin production. Thus not only does NO exert an inhibitory effect on prostacyclin production in vitro (32), but also enhanced prostacyclin production maintains flow-mediated dilation in endothelial NO synthase (eNOS) knockout mice (42). Furthermore, inhibition of cyclooxygenase (COX) affected the duration of reactive hyperemia in dogs treated with N-nitro-L-arginine methyl ester but not control dogs (38). We have previously shown that, whereas endogenous NO exerts a vasodilator influence on the systemic, pulmonary, and coronary vasculature of awake swine, the exercise-induced vasodilation was only slightly blunted after inhibition of NO production (9). Hence, other vasodilators, such as prostacyclin, could have an increased contribution to exercise-induced vasodilation and act to compensate when eNOS activity is blunted. The second aim of the present study was therefore to determine whether the contribution of prostacyclin in the regulation of resistance vessel tone is enhanced after inhibition of eNOS in exercising swine.

METHODS

Animals

Studies were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 86-23, Revised 1996) and with the approval of the Animal Care Committee of Erasmus Medical Center. Fifteen 2- to 3-mo-old Yorkshire × Landrace swine (23 ± 1 kg at the time of surgery) of either sex (4 males and 11 females) entered the study. Daily adaptation of the animals to laboratory conditions started 1 wk before surgery. Experiments were started 10 days after surgery.

Surgery

Swine were sedated (20 mg/kg im ketamine), anesthetized (10 mg/kg iv thiopental), intubated, and ventilated with O\textsubscript{2} and N\textsubscript{2}O, to which 0.2–1% (vol/vol) isoflurane was added (9, 10, 12). Anesthesia was maintained with midazolam (2 mg/kg followed by 1 mg/kg \textsuperscript{-1}h\textsuperscript{-1} iv) and fentanyl (10 \mu g/kg \textsuperscript{-1}h\textsuperscript{-1} iv). Under sterile conditions, the chest was opened via the fourth left intercostal space, and a fluid-filled polyvinyl chloride catheter was inserted into the pulmonary artery.
aortic arch for aortic blood pressure measurement (Combitrans pressure transducers, Braun) and blood sampling for determination of blood gases (Acid-Base Laboratory model 505, Radiometer), O₂ saturation and hemoglobin concentration (OSM2, Radiometer), and computation of O₂ content, O₂ supply, and O₂ consumption (9, 10, 12). An electromagnetic flow probe (14–15 mm, Skalar) was positioned around the ascending aorta for measurement of cardiac output. A microtipped pressure-transducer (P4.5, Konigsberg Instruments) was inserted into the left ventricle (LV) via the apex. Polyvinylchloride catheters were inserted into the LV to calibrate the Konigsberg transducer LV pressure signal, and into the left atrium to measure pressure and inject radioactive microspheres to determine regional blood flows (9). Catheters were inserted into the pulmonary artery to measure pressure, administer drugs, and collect mixed venous samples. An angiocatheter was inserted into the anterior interventricular vein for blood sampling (9, 10, 12) while a Transonic flow probe (2.5–3.0 mm, Transonic Systems) was placed around the left anterior descending (LAD) coronary artery. Catheters were tunneled to the animal’s back, and animals were allowed to recover and received analgesia (0.3 mg buprenorphine im) for 2 days and antibiotic prophylaxis (25 mg/kg amoxicillin and 5 mg/kg gentamycin iv) for 5 days.

Experimental Protocols

**Dosage and stability of the effect induced by indomethacin.** To validate that the employed dose of indomethacin (10 mg/kg iv dissolved in 20 ml saline; pH 9.0) produced complete COX inhibition, we studied the hemodynamic effects of 1 and 10 mg/kg iv indomethacin in 11 resting swine (27 ± 1 kg). After baseline measurements of heart rate, blood pressure, and cardiac output had been obtained, animals received a dose of 1 mg/kg iv indomethacin, and 5 min later hemodynamic measurements were repeated. Animals then received additional indomethacin to achieve a total dose of 10 mg/kg iv, and 5 min after the administration was completed measurements were again repeated.

To validate the stability of COX inhibition by indomethacin, i.e., whether the degree of COX inhibition was maintained during the entire 15-min exercise protocol, we studied the stability of the hemodynamic responses to indomethacin (10 mg/kg iv) in four resting swine over a 20-min period. For this purpose, hemodynamic baseline measurements were obtained, and animals received indomethacin administered over a 10-min period. Five minutes after the completion of administration, hemodynamics were measured again over a 20-min period.

**Role of prostanoids in the regulation of vasomotor tone.** Systemic, pulmonary, and coronary hemodynamic responses to exercise were studied in 13 swine (27 ± 1 kg). After baseline hemodynamic measurements (lying and standing), blood samples (lying), and temperature (standing) were obtained, a treadmill exercise protocol was begun (1–5 km/h); hemodynamic data and blood samples were collected during the last 30 s of each 3-min exercise stage (9, 10, 12). After the completion of the exercise protocol, animals were allowed to rest for 90 min. Subsequently, indomethacin (10 mg/kg) was administered intravenously over a 10-min period, and 5 min later the exercise protocol was repeated. We have previously shown excellent reproducibility of hemodynamic responses to consecutive exercise bouts (10, 12).

On another day, six swine underwent a control exercise trial, and, after 90 min of rest, animals received the COX inhibitor ibuprofen (60 mg/kg iv) and underwent a second trial.

**Regional blood flows.** On a different day, regional blood flows were determined in four swine using the radioactive microsphere technique (9). Radioactive microspheres were injected at rest (lying) and during exercise at 5 km/h under control conditions as well as 5 min after the administration of indomethacin (10 mg/kg iv).

### Role of prostanoids in the regulation of vasomotor tone in the presence of NOS inhibition.

On a different day, seven animals (29 ± 2 kg) underwent a control exercise trial, and, after 90 min of rest, L⁷⁷-nitro-L-arginine (L-NNA; 20 mg/kg dissolved in 100 ml saline) was administered intravenously over a 20-min period, which was followed 10 min later by a second exercise trial. After another 90 min of rest, animals received indomethacin (10 mg/kg iv) and underwent a third exercise trial.

### Data Analysis

Digital recording and off-line analysis of hemodynamics and regional blood flow have been described previously (9, 10, 12). Systemic vascular conductance was computed as cardiac output divided by mean aortic blood pressure. Systemic vascular resistance was computed as mean aortic blood pressure divided by cardiac output. Pulmonary vascular resistance is defined as mean pulmonary artery pressure minus mean pulmonary backpressure divided by cardiac output. Pulmonary backpressure is best reflected in the pulmonary capillary wedge pressure, but because the increases in left atrial pressure that we observed in our laboratory in exercising swine (Refs. 9, 10, 12 and present study) agree very well with the reported increases in pulmonary capillary wedge pressure (from 4 mlHg at rest to 11 mlHg during exercise at comparable increases of heart rate (22)), we employed mean left atrial pressure as backpressure. Blood O₂ content (in μmol/ml) was calculated as (Hb-0.621–O₂ saturation) + (0.00131–P₅₀). Body O₂ consumption (BVO₂) was calculated as the product of cardiac output and the difference in O₂ content between arterial and mixed venous blood. Myocardial O₂ delivery (MDO₂) was computed as the product of LAD coronary blood flow and arterial blood O₂ content. Myocardial O₂ consumption (MVO₂) in the region of myocardium perfused by the LAD coronary artery was calculated as the product of coronary blood flow and the difference in O₂ content between arterial and coronary venous blood. Myocardial O₂ extraction (MEO₂) was computed as the ratio of MVO₂ and MD0₂.

### Statistical Analysis

Statistical analysis of hemodynamic data within the separate study protocols (control and indomethacin; control and ibuprofen; and control, L-NNA, and L-NNA + indomethacin) was performed using two-way (treatment and exercise level) analysis of variance (ANOVA) for repeated measures. When a significant effect was detected, post hoc testing for exercise and drug effects was performed using Scheffé’s test.

To test for the effects of indomethacin and L-NNA on the relation between VO₂ and hemodynamic and metabolic variables, analysis of covariance (ANCOVA) was performed in the separate study protocols (control and indomethacin; and control, L-NNA, and L-NNA + indomethacin) using treatment as an independent factor and VO₂ as the covariate. Post hoc testing for drug effects (i.e., control vs. L-NNA and L-NNA vs. L-NNA + indomethacin) was performed using Scheffe’s test.

### Table 1. Hemodynamic effect of 1 vs. 10 mg/kg Indo in resting swine

<table>
<thead>
<tr>
<th>HR, beats/min</th>
<th>Baseline</th>
<th>1 mg/kg iv</th>
<th>10 mg/kg iv</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>83±5</td>
<td>83±5</td>
<td>135±5*</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>3.4±0.2</td>
<td>2.8±0.2*</td>
<td>2.6±0.2*</td>
</tr>
<tr>
<td>SVR, mmHg·1·min⁻¹</td>
<td>28±2</td>
<td>47±3*</td>
<td>51±7*</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 11 swine. Indo, indomethacin; HR, heart rate; MAP, mean arterial pressure; CO, cardiac output; SVR, systemic vascular resistance. *P ≤ 0.05 vs. baseline; †P ≤ 0.05 vs. 1 mg/kg iv indomethacin.
Table 2. Stability of Indo (10 mg/kg iv) induced hemodynamic alterations in resting swine

<table>
<thead>
<tr>
<th>Time After Start of Protocol, min</th>
<th>Baseline</th>
<th>0</th>
<th>10</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>133 ± 4</td>
<td>92 ± 6*</td>
<td>93 ± 7*</td>
<td>93 ± 7*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>89 ± 2</td>
<td>117 ± 5*</td>
<td>112 ± 5*†</td>
<td>113 ± 5*†</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>3.5 ± 0.4</td>
<td>2.6 ± 0.3*</td>
<td>2.7 ± 0.3*</td>
<td>2.8 ± 0.3*</td>
</tr>
<tr>
<td>SVR, mmHg/L·min</td>
<td>27 ± 4</td>
<td>50 ± 10*</td>
<td>47 ± 9*†</td>
<td>45 ± 9*†</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 4 swine. *P ≤ 0.05 vs. baseline; †P ≤ 0.05 vs. 0 min start time.

To test for the interaction of the treatments (indomethacin × L-NNa) on the relation between VO2 and hemodynamic and metabolic variables, ANCOVA was performed using indomethacin and L-NNa as two independent factors and VO2 as the covariate. Statistical significance was accepted when P ≤ 0.05. Data are presented as means ± SE.

RESULTS

Dosage and Stability of the Effect Induced by Indomethacin

The increase in mean aortic blood pressure and systemic vascular resistance produced by 1 mg/kg indomethacin was identical to the vasoconstrictor and pressure response to 10 mg/kg indomethacin, although heart rate decreased somewhat less after 1 than 10 mg/kg (Table 1). These findings indicate that the dose of 10 mg/kg indomethacin, which we employed in the exercise experiments, produced a maximal effect. In addition, the effect of indomethacin was well maintained over a 20-min period (<10% change; Table 2).

Hemodynamic Effects of COX Inhibition in Exercising Swine

Systemic and pulmonary circulation. Exercise up to 5 km/h resulted in a more than doubling of cardiac output, which was principally due to the increase in heart rate (up to 85% of maximum heart rate), as stroke volume increased by only 15% (Table 3). Despite the increase in cardiac output, mean aortic blood pressure was maintained, reflecting marked systemic vasodilation, i.e., a doubling of systemic vascular conductance or a 60% decrease in systemic vascular resistance (Fig. 1).

Administration of indomethacin resulted in a 40% increase in aortic blood pressure, due to systemic vasoconstriction (Table 3 and Fig. 1). The accompanying decrease in cardiac output was mediated by a (probably baroreflex-mediated) decrease in heart rate (Table 3). The indomethacin-induced systemic vasoconstriction necessitated an increase in O2 extraction, resulting in a decreased mixed venous O2 saturation (Fig. 1). The pressor and vasoconstrictor responses to indomethacin were progressively blunted during exercise (Table 3 and Fig. 1). That the increase in systemic vascular resistance was progressively blunted during exercise was not merely the

Table 3. Hemodynamic parameters before and after administration of Indo

<table>
<thead>
<tr>
<th></th>
<th>Lying</th>
<th>Standing</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
<td></td>
<td>1 Rest Exercise Level, km/h</td>
<td>2</td>
<td>3</td>
<td>4</td>
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<tr>
<td>CO, l/min</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.4 ± 0.2</td>
<td>4.3 ± 0.3*</td>
<td>5.3 ± 0.2*</td>
<td>5.8 ± 0.3*</td>
<td>6.3 ± 0.3*</td>
<td>7.1 ± 0.3*</td>
<td>8.1 ± 0.3*</td>
</tr>
<tr>
<td>Indo</td>
<td>2.6 ± 0.2†</td>
<td>3.5 ± 0.2ª</td>
<td>4.8 ± 0.2ª</td>
<td>4.8 ± 0.3ª†</td>
<td>5.5 ± 0.3ª†</td>
<td>6.5 ± 0.4ª†</td>
<td>7.4 ± 0.3ª†</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Control</td>
<td>121 ± 5</td>
<td>139 ± 6*</td>
<td>167 ± 5ª</td>
<td>180 ± 5ª</td>
<td>201 ± 6*</td>
<td>229 ± 7ª</td>
<td>254 ± 4ª</td>
</tr>
<tr>
<td>Indo</td>
<td>87 ± 5†</td>
<td>103 ± 4ª</td>
<td>119 ± 4ª</td>
<td>128 ± 3ª†</td>
<td>148 ± 5ª†</td>
<td>174 ± 6ª†</td>
<td>203 ± 6ª†</td>
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<tr>
<td>SV, ml</td>
<td></td>
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<tr>
<td>Control</td>
<td>28 ± 2</td>
<td>32 ± 2ª</td>
<td>32 ± 2ª</td>
<td>32 ± 2ª</td>
<td>31 ± 2ª</td>
<td>32 ± 1ª</td>
<td>32 ± 1ª</td>
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<tr>
<td>Indo</td>
<td>30 ± 2</td>
<td>34 ± 2ª</td>
<td>36 ± 2ª</td>
<td>37 ± 2ª</td>
<td>37 ± 1ª</td>
<td>37 ± 1ª</td>
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<tr>
<td>LV dp/dt max, mmHg/s</td>
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<td></td>
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<tr>
<td>Control</td>
<td>2,550 ± 150</td>
<td>2,910 ± 190*</td>
<td>3,490 ± 210*</td>
<td>3,710 ± 180*</td>
<td>4,280 ± 280*</td>
<td>4,770 ± 350*</td>
<td>5,460 ± 370*</td>
</tr>
<tr>
<td>Indo</td>
<td>2,410 ± 210</td>
<td>3,060 ± 230*</td>
<td>3,050 ± 190*†</td>
<td>3,170 ± 240*†</td>
<td>3,630 ± 260*†</td>
<td>4,240 ± 320*</td>
<td>4,870 ± 390*†</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>93 ± 2</td>
<td>89 ± 3</td>
<td>85 ± 3ª</td>
<td>86 ± 3ª</td>
<td>87 ± 3ª</td>
<td>89 ± 3</td>
<td>91 ± 3</td>
</tr>
<tr>
<td>Indo</td>
<td>132 ± 6†</td>
<td>124 ± 7ª†</td>
<td>111 ± 5ª†</td>
<td>109 ± 5ª†</td>
<td>108 ± 5ª†</td>
<td>103 ± 5ª†</td>
<td>102 ± 4ª†</td>
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<td>MPAP, mmHg</td>
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<tr>
<td>Control</td>
<td>14 ± 1</td>
<td>14 ± 1</td>
<td>17 ± 2ª</td>
<td>19 ± 2ª</td>
<td>22 ± 2ª</td>
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<tr>
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<td>18 ± 2</td>
<td>20 ± 2</td>
<td>22 ± 2</td>
<td>25 ± 2</td>
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</tr>
<tr>
<td>Control</td>
<td>2 ± 1</td>
<td>2 ± 1</td>
<td>0 ± 1</td>
<td>3 ± 1</td>
<td>5 ± 1ª</td>
<td>8 ± 1ª</td>
<td>10 ± 1ª</td>
</tr>
<tr>
<td>Indo</td>
<td>13 ± 1†</td>
<td>8 ± 1ª†</td>
<td>6 ± 2ª</td>
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<td>9 ± 1ª</td>
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<tr>
<td>CBF, ml/min</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>56 ± 4</td>
<td>73 ± 6ª</td>
<td>81 ± 6ª</td>
<td>87 ± 7ª</td>
<td>99 ± 7ª</td>
<td>116 ± 10ª</td>
<td>140 ± 11ª</td>
</tr>
<tr>
<td>Indo</td>
<td>49 ± 5†</td>
<td>60 ± 5ª†</td>
<td>66 ± 5ª†</td>
<td>71 ± 6ª†</td>
<td>81 ± 8ª</td>
<td>98 ± 11ª†</td>
<td>114 ± 11ª†</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 13 swine. SV, stroke volume; LV dp/dt max, rate of increase in left ventricular pressure; MPAP, mean pulmonary artery pressure; MLAP, mean left atrial pressure; CBF, coronary blood flow. *P ≤ 0.05 vs. rest (lying); †P ≤ 0.05 vs. control.
mathematical result of a smaller absolute effect of indomethacin at lower resistance values, because the relative increase in resistance in response to indomethacin was also greater at rest than during exercise (Fig. 2). Although the absolute indomethacin-induced decrease in systemic conductance was similar at rest and during exercise, the relative decrease in conductance induced by indomethacin was also significantly greater at rest than during exercise (Fig. 2). Furthermore, the effects of indomethacin on body O2 extraction and mixed venous O2 saturation (Fig. 1) also waned during exercise. Thus the slope of the relation between body O2 consumption and O2 extraction decreased from 1.13 $\pm$ 0.07 under control conditions to $-1.06 \pm 0.07$ under control conditions to $-0.73 \pm 0.10$ in the presence of indomethacin ($P < 0.05$).

In six animals, ibuprofen caused systemic vasoconstriction, as evidenced by significant increases in blood pressure (from 91 $\pm$ 4 to 102 $\pm$ 4 mmHg, $P < 0.05$) and systemic vascular resistance (from 30 $\pm$ 2 to 35 $\pm$ 4 mmHg$^{-1}$min$^{-1}$, $P < 0.05$), albeit to a lesser extent than indomethacin. Similar to indomethacin, the effect of ibuprofen waned during exercise (at maximal exercise: blood pressure 90 $\pm$ 4 mmHg without ibuprofen and 94 $\pm$ 4 mmHg with ibuprofen; systemic vascular resistance 15 $\pm$ 2 mmHg$^{-1}$min$^{-1}$ without ibuprofen and 17 $\pm$ 2 mmHg$^{-1}$min$^{-1}$ with ibuprofen).

Pulmonary artery pressure more than doubled during exercise (Table 3), whereas pulmonary vascular resistance decreased by only 25% (Fig. 3). In contrast to the systemic...
vasculature, indomethacin had no effect on pulmonary vascular resistance either at rest or during exercise (Fig. 3).

**Regional systemic vascular beds.** The exercise-induced increase in cardiac output was principally diverted toward skeletal muscle, and, although brain flow increased slightly, flow to most visceral organs decreased (Fig. 4). Vasoconstriction in response to indomethacin occurred in most visceral organs, such as the kidneys and intestine (with the exception of the adrenals and spleen), and various regions of the brain (Fig. 4). In contrast, skeletal muscle flow was not altered by indomethacin either at rest or during exercise.

**Coronary circulation.** Exercise resulted in an increase in MVO<sub>2</sub> that was matched by an equivalent increase in coronary blood flow and hence MDO<sub>2</sub>, so that MEO<sub>2</sub> and coronary venous PO<sub>2</sub> (cvPO<sub>2</sub>) were maintained constant over the entire range of MVO<sub>2</sub> (Fig. 5). Indomethacin reduced coronary blood flow at any given level of MVO<sub>2</sub>, necessitating an increase in MEO<sub>2</sub> (to maintain MVO<sub>2</sub>), which resulted in a decrease in cvPO<sub>2</sub>. The increase in coronary blood flow was blunted in the

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**Fig. 3.** Effect of inhibition of prostanoid synthesis with Indo (10 mg/kg iv) on the relation between exercise and pulmonary vascular resistance (PVR) in the absence (A) and presence (B) of NO synthase inhibition with L-NNA (20 mg/kg iv). Note that Indo had no effect on PVR. Dots inside symbols denote changes (P ≤ 0.05) from resting (lying) measurements; *P ≤ 0.05 vs. control (ANOVA).

**Fig. 4.** Effect of inhibition of prostanoid synthesis with Indo (10 mg/kg iv) on visceral organ, regional brain, and skeletal muscle blood flows at rest (lying) and during exercise at 5 km/h. Dots inside symbols denote changes (P ≤ 0.05) from resting (lying) measurements; *P ≤ 0.05 vs. control relation; †P ≤ 0.05, effect of Indo waned during exercise.
presence of indomethacin ($P < 0.05$), suggesting that prostanoids may contribute to metabolic dilation in the coronary vasculature. However, the indomethacin-induced increase in ME$O_2$ and decrease in CV$P_O_2$ were not significantly different during exercise compared with resting conditions (Fig. 5), indicating that the relative contribution of prostanoids to coronary vascular tone was similar at rest and during exercise.

**Hemodynamic Effects of COX Inhibition in Exercising Swine with Inhibited NOS Activity**

**Systemic and pulmonary circulation.** NOS inhibition with L-NNA produced vasoconstriction in the systemic circulation, resulting in an increase in aortic blood pressure (Fig. 1 and Table 4). Importantly, pretreatment with L-NNA enhanced the indomethacin-induced vasoconstriction in the systemic circulation, as indicated by the exaggerated increase in systemic vascular resistance and mixed venous $O_2$ saturation (Fig. 1). Although the absolute indomethacin-induced decreases in systemic vascular conductance were not augmented (Fig. 1), the relative changes in systemic vascular conductance and resistance both showed potentiation of the vasoconstrictor effect of indomethacin by L-NNA, particularly at rest (Fig. 2). Despite the marked potentiation of the systemic vasoconstrictor response to indomethacin by L-NNA, the exercise-induced systemic vasoconstriction was unmitigated.

NOS inhibition produced vasoconstriction in the pulmonary circulation (Fig. 3), resulting in an increase in pulmonary artery pressure (Table 4). However, subsequent administration of indomethacin had no additional effect on pulmonary vascular resistance (Fig. 3).

**Coronary circulation.** L-NNA reduced coronary blood flow and MDO$_2$ at any given level of MVO$_2$, necessitating a small increase in ME$O_2$ (to maintain MVO$_2$), which resulted in a decrease in CV$P_O_2$ (Fig. 5). Although indomethacin resulted in additional vasoconstriction as evidenced by a further decrease in CV$P_O_2$, pretreatment with L-NNA did not enhance the vasoconstrictor effect of indomethacin, indicating that increased prostanoid production does not compensate for the loss of NO in the coronary circulation.

**DISCUSSION**

The major findings of the present study are that in awake swine free from the effects of anesthesia and acute surgical trauma 1) prostanoids are involved in the regulation of resistance vessel tone in the systemic and coronary circulation, but not in the pulmonary circulation; 2) the vasodilator influence of prostanoids on coronary and cerebral resistance vessels is maintained during exercise, whereas their influence on the total systemic vasculature wanes during exercise; and 3) prostanoids have an increased vasodilator influence in the systemic vascular bed when eNOS activity is inhibited.

**Methodological Considerations**

**Dosage of indomethacin.** The dose of indomethacin that we used (10 mg/kg iv) is similar (45, 46) or slightly higher [compared with 3 mg/kg iv (1, 5)] than that used by others to inhibit COX in swine. This dose should be sufficient to completely block COX for a prolonged period of time in swine because in the present study administration of indomethacin at a dose of 1 mg/kg yielded similar alterations in hemodynamic variables at rest. To exclude that the decreased effects of indomethacin on the systemic and coronary vasculature during exercise were due to diminished blockade of COX, we estab-
may be affected (43). We therefore performed additional ex-
periments to elucidate the mechanisms by which COX inhibitors have other effects besides inhibition of COX. Depending on the inhibitor used, transcription factors, MAPKs, cell cycle proteins, and heat shock proteins (HSPs) were examined. Our findings suggest that COX inhibition resulted in net vasoconstriction, which may have quantitatively nullified the vasodilator effects of other prostanoids. Thus the decreased effect of indomethacin during exercise is likely due to a decreased contribution of endogenous prostanoids to the regulation of vascular tone or an increased compensation by other vasodilator systems during exercise, as will be discussed below.

**Indomethacin as a COX inhibitor.** Some studies have indicated that COX inhibitors have other effects besides inhibition of COX. Depending on the inhibitor used, transcription factors, MAPKs, cell cycle proteins, and heat shock proteins (HSPs) may be affected (43). We therefore performed additional experiments with a different COX inhibitor (ibuprofen). Ibuprofen also causes systemic vasoconstriction, albeit to a lesser extent than indomethacin. Similar to indomethacin, the effect of ibuprofen waned during exercise. One of the possible differences between ibuprofen and indomethacin is that ibuprofen activates HSP formation, whereas indomethacin is devoid of such action (43). Increased HSP90 expression may subsequently activate eNOS (20, 21, 24), thereby increasing NO production and partially counterbalancing the effect of the loss of the vasodilator prostanoids. However, the observation that indomethacin and ibuprofen induce qualitatively similar effects on systemic hemodynamic parameters suggests that the effects of indomethacin on cardiovascular function in the present study are indeed due to inhibition of COX. COX catalyzes the conversion of arachidonic acid into prostaglandin H2, which is subsequently processed by different enzymes into various prostanoids. The vasodilator prostacyclin and vasoconstrictor thromboxane are the most vasoactive prostanoids, and because COX inhibition resulted in net vasoconstriction it is likely that inhibition of prostacyclin was responsible for the observed vasoconstriction.

**Age of the animals.** The animals used in the present study were young adults (3 to 4 mo), which may have quantitatively influenced the contribution and interaction between NO and prostanoids. However, the contribution of prostanoids in both

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**Table 4. Hemodynamic parameters before and after administration of L-NNA and Indo**

<table>
<thead>
<tr>
<th></th>
<th>Lying</th>
<th>Standing</th>
<th>Exercise Level, km/h</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systemic hemodynamics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO, l/min</td>
<td>3.8±0.4</td>
<td>4.8±0.4*</td>
<td>5.5±0.4</td>
<td>6.1±0.5*</td>
<td>6.6±0.5*</td>
<td>7.3±0.5*</td>
<td>8.1±0.6*</td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>1.9±0.2*</td>
<td>2.6±0.2*†</td>
<td>3.3±0.3*†</td>
<td>3.7±0.3*†</td>
<td>4.2±0.3*†</td>
<td>4.2±0.4*†</td>
<td>6.0±0.4*†</td>
<td></td>
</tr>
<tr>
<td>SV, ml</td>
<td>32±3</td>
<td>35±3</td>
<td>36±3*</td>
<td>34±3</td>
<td>33±2</td>
<td>33±2</td>
<td>33±2</td>
<td></td>
</tr>
<tr>
<td>LV dp/dt max, mmHg/s</td>
<td>2.960±200</td>
<td>3.220±260</td>
<td>3.500±180*</td>
<td>4.050±270*</td>
<td>4.330±270*</td>
<td>4.820±420*</td>
<td>5.280±430*</td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>93±2</td>
<td>87±5</td>
<td>83±3*</td>
<td>86±3</td>
<td>91±2</td>
<td>92±3</td>
<td>94±3</td>
<td></td>
</tr>
<tr>
<td>MPAP, mmHg</td>
<td>15±1</td>
<td>17±1</td>
<td>18±1*</td>
<td>21±1*</td>
<td>27±1*</td>
<td>30±2*</td>
<td>35±2*</td>
<td></td>
</tr>
<tr>
<td>MLAP, mmHg</td>
<td>22±2†</td>
<td>21±3</td>
<td>25±3†</td>
<td>28±3†</td>
<td>33±4†</td>
<td>39±4†</td>
<td>42±3†</td>
<td></td>
</tr>
<tr>
<td>MLAP, mmHg</td>
<td>29±2†</td>
<td>26±2†</td>
<td>29±2†</td>
<td>31±2†</td>
<td>35±2†</td>
<td>39±2†</td>
<td>41±3†</td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>3±1</td>
<td>3±2</td>
<td>3±2</td>
<td>3±2</td>
<td>9±2*</td>
<td>11±2*</td>
<td>14±2*</td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>10±2</td>
<td>4±3</td>
<td>7±2†</td>
<td>7±2†</td>
<td>11±1</td>
<td>12±1</td>
<td>14±2</td>
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<tr>
<td>MAP, mmHg</td>
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<td>12±3</td>
<td>14±2</td>
<td>15±2</td>
<td>16±3</td>
<td>17±3</td>
<td>16±2</td>
<td></td>
</tr>
</tbody>
</table>

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Data are means ± SE; n = 7 swine. L-NNA, Nω-nitro-L-arginine. *P ≤ 0.05 vs. rest (lying); †P ≤ 0.05 vs. control; ‡P ≤ 0.05 vs. 1. N-NAA. For abbreviations, see Table 3.
basal vascular tone as well as vasodilator responses to agonists is independent of age (36) or may decrease slightly from newborn to young adult swine (46, 47). In contrast, the role of NO in the regulation of tone in the cerebral vasculature does vary with age. However, the largest difference is found between newborn and young adult swine, so that whereas a contribution of NO is absent in newborn piglets, its importance increases in young adult and adult swine (36, 46, 47). In accordance with these reports, we observed in the young adult swine in the present study that NO contributed importantly to regulation of vascular tone. Taken together, it seems that the swine were of sufficient maturity so that their age did not quantitatively influence the results.

Role of Prostanoids in the Regulation of Vascular Tone

Systemic circulation. Endogenous prostanoids exerted a strong vasodilator influence on the systemic vasculature at rest, as was evidenced by the marked increase in blood pressure and the increase in systemic vascular resistance upon indomethacin administration. Although prostanoids contribute importantly to the regulation of vascular tone at rest, blood pressure and systemic vascular resistance gradually returned to control levels during exercise and mixed venous \( O_2 \) saturation approached the values of the control exercise. These findings indicate that either there is no role of prostanoids in metabolic regulation or that their role in the systemic vascular bed is fully compensated by other vasoactive substances such as NO. Little is known about the role of prostanoids in the systemic vasculature during exercise. Because prostacyclin is produced in response to increased levels of adrenaline (40), prostacyclin production will likely increase during exercise, when epinephrine levels increase. Indeed, prostacyclin production increases in the human leg during exercise (14, 18, 23). Furthermore, Sun et al. (42) showed that prostacyclin is involved in flow-dependent dilation of isolated murine skeletal muscle arterioles but did not find a contribution of prostacyclin to basal tone in these vessels. These findings suggest that prostanoids could indeed have increased importance during exercise and thereby contribute to metabolic vasodilatation. In apparent support of this concept, Duffy et al. (8) reported that prostanoids contribute to exercise-induced vasodilatation in the human forearm. However, in forearm exercise studies, flow is measured immediately after rather than during exercise, which may have influenced the results. For example, inhibition of NO has been shown to reduce blood flow in the recovery phase after exercise but not during exercise (39). In line with this view, Beaty et al. (4) showed in anesthetized dogs that inhibition of prostanoic synthesis decreased flow to resting skeletal muscle to an equal extent as skeletal muscle flow during electrical stimulation. The present study in awake swine demonstrates that inhibition of prostanoic production does not affect flow to various skeletal muscle groups with different fiber type composition (9) either at rest or during exercise up to 85% of maximum heart rate.

The present study also shows that the role of prostanoids is strongly dependent on the vascular bed. Inhibition of prostanoic production resulted in a decrease in flow to various regions of the brain of almost 50%, both at rest and during exercise. However, indomethacin did not alter the exercise-induced vasodilation, suggesting that prostanoids are not essential for metabolic vasodilation in the various regions of the brain. Flow to the kidneys and most other visceral organs was also decreased by indomethacin in resting swine, indicating that prostanoids are important for maintaining basal flow to visceral organs. During exercise, however, flow is redistributed away from the visceral organs toward the exercising skeletal muscle. The observation that the effect of indomethacin decreased during exercise suggests that this redistribution may in part be caused by withdrawal of a prostanoid-mediated vasodilator influence in the visceral organs. Conversely, the decreased effect of indomethacin on the visceral organs during exercise is translated into a decreased effect on total systemic vascular resistance, blood pressure, and mixed venous \( O_2 \) saturation, because flow to skeletal muscle is not affected by indomethacin either at rest or during exercise.

We found in the present study that the effect of inhibition of prostanoic production in the systemic circulation was larger after inhibition of NO synthesis compared with the control experiments, indicating that prostanoids compensate in part for the loss of NO synthesis. There is evidence that NO directly suppresses the activity of COX in vitro (28, 31, 32) as well as in vivo. Prostanoids only contribute to the regulation of coronary tone in dogs after chronic inhibition of NOS (38) as well as in collateral-dependent myocardium (2, 3). Also, prostacyclin is important in the regulation of flow-induced dilation in eNOS knockout mice (42). Functionally, it is important to maintain vasodilator capacity through redundant or backup mechanisms. NO and prostacyclin production are both activated in response to physical stimuli such as shear stress (25, 44), as well as agonists such as acetylcholine and bradykinin (44), and share similar regulatory mechanisms such as activation of COX and NOS by tyrosine phosphorylation (35) and increased intracellular calcium concentrations (27). Consequently, prostacyclin is a good candidate to act as a backup system in the systemic circulation where NO bioavailability is reduced.

Pulmonary circulation. We found that prostanoids do not play a role in the regulation of pulmonary vascular tone at rest in either the presence or absence of NO. This is in accordance with a study from Albertini et al. (1), who showed that endogenous prostanoids do not contribute to pulmonary vascular tone in anesthetized swine in either the presence or absence of eNOS inhibition. In accordance with a previous study from our laboratory (9), the modest exercise-induced pulmonary vasodilation was principally mediated by NO. However, even when NO production was inhibited, there was no role for endogenous prostanoids in the regulation of exercise-induced pulmonary vasodilation. Thus, the porcine pulmonary conductance and resistance vessels are sensitive to the vasodilator actions of exogenous prostanoids (1, 29, 33), endogenous prostanoids do not appear to exert a significant vasodilator influence in the pulmonary circulation.

Coronary circulation. The normal heart is characterized by a high level (80%) of ME\( O_2 \) under basal resting conditions (16, 26). 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 supply of \( O_2 \). A sensitive way to study alterations in coronary vascular tone in relation to myocardial metabolism is the relationship between cv\( P_0 \) and MVO\(_2\). For example, an increase in coronary resistance vessel tone will limit coronary blood flow and...
hence $\text{MDo}_2$, forcing the myocardium to increase its $\text{MEO}_2$ (to maintain $\text{MVO}_2$), which results in a lower $\text{cvPO}_2$. The $\text{cvPO}_2$ thus represents an index of myocardial tissue oxygenation (i.e., the balance between $\text{MDo}_2$ and $\text{MVO}_2$), which is principally determined by coronary resistance vessel tone.

In the coronary vasculature, we found prostanoids to be involved in the regulation of vascular tone both at rest and during exercise, which is in accordance with most (7, 13, 19, 34), but not all (15) data from the human coronary circulation. Prostanoids have been proposed to contribute to metabolic dilation of the coronary resistance vessels in humans (7, 19), although this is not a ubiquitous finding (15, 34). The present study demonstrates that in swine prostanoids do not appear to have increased importance during exercise compared with resting conditions. In contrast to humans and swine, prostanoids do not appear to be important in the regulation of coronary vascular tone at rest or during exercise in the dog (6). Although the different observations are difficult to reconcile, they may be attributable to variations in preferential vasodilator systems between species. The contribution of prostanoids to the regulation of coronary vascular tone was not altered by inhibition of NO synthesis. These findings suggest that prostanoids and NO do not act in a compensatory manner when the other pathway is blocked. However, we cannot entirely exclude that the near-maximal $\text{MEO}_2$ (91–93% of the $\text{O}_2$ supplied) that occurred after combined blockade of NO and prostanoid synthesis prevented detection of an interaction between NO and prostanoids.

Metabolic Vasodilation After Inhibition of NO and Prostanoids

Although blockade of production of NO and prostanoids results in severe vasoconstriction at rest and during exercise, exercise-induced vasodilation was principally unperturbed as evidenced by the increase in coronary blood flow and the decrease in systemic vascular resistance during exercise. There are several vasoreactive factors that could have contributed to this exercise-induced vasodilation, including adenosine and ATP-sensitive $K^+$ channels (30) and $\beta$-adrenergic vasodilation (10, 41). In addition, EDHF may have contributed because it has been shown to be released in response to various agonists (17) as well as pulsatile stress (37) and may thus have increased importance at higher heart rates, which occur during exercise.

In conclusion, the present study demonstrates that the contribution of prostanoids to vasomotor control varies markedly between regional vascular beds. Thus endogenous prostanoids do not play a role in pulmonary resistance vessel control. Conversely, prostanoids contribute significantly to vasomotor and blood flow control in the brain and heart both at rest and during exercise but are not mandatory for the exercise-induced vasodilation in these organs. In contrast, prostanoids do not appear to play a role in skeletal blood flow regulation either at rest or during exercise. In most visceral organs, prostanoids exert a marked vasodilator influence under resting conditions that is withdrawn during exercise. This exercise-induced withdrawal of prostanoid-mediated vasodilation may contribute to the exercise-induced redistribution of blood flow from the visceral organs toward the active skeletal muscle. Finally, prostanoids compensate for an acute loss of NO in the systemic circulation but not in the coronary and pulmonary circulation.

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