Cardiovascular actions of ET-B activation in vivo and modulation by receptor antagonism

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Cardiovascular Research Laboratory, Divisions of 1Cardiovascular Disease and 2Vascular Surgery, Departments of Internal Medicine and Surgery, Mayo Clinic, Rochester, Minnesota 55905; and 3SmithKline Beecham, King of Prussia, Pennsylvania 19406

Rasmussen, Todd E., Michihisa J Ougasaki, Thanom Supaporn, John W. Hallett, Jr., David P. Brooks, and John C. Burnett, Jr. Cardiovascular actions of ET-B activation in vivo and modulation by receptor antagonism. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R131–R138, 1998.—The endothelin (ET)-B receptor subtype is expressed on vascular endothelial and smooth muscle cells and participates in vasodilatation and vasoconstriction. Controversy exists regarding the role of the ET-B receptor as a mediator of systemic, pulmonary, and renal vasoconstriction in states of marked ET-1 activation. Moreover, the potential activation of endogenous ET-1 with secondary stimulation of the ET-A receptor in response to sarafotoxin S6c (S6c) remains unclear. This study was designed to assess the cardiovascular actions of ET-B activation with S6c in the presence and absence of selective ET-A antagonism with FR-139317 and dual ET-A/ET-B antagonism with SB-209670 in the anesthetized dog. Compared with time control (n = 5), S6c increased from baseline systemic vascular resistance (SVR) [28 ± 7 vs. 14 ± 3 resistance units (RU), P < 0.05] and pulmonary vascular resistance (PVR) (3.2 ± 0.7 vs. 0.9 ± 0.3 RU, P < 0.05) and decreased cardiac output (CO) (−1.7 ± 0.3 vs. −0.5 ± 0.1 l/min, P < 0.05), with no differences in renal vascular resistance in association with increases in plasma ET-1. S6c also decreased mixed venous oxygen saturation (SVO2) (56 ± 6 vs. 76 ± 5%, P < 0.05). Selective ET-A receptor antagonism did not affect the actions of S6c, with the exception that ET-A receptor antagonism blocked the increase in SVR to high-dose S6c. Dual ET-A/ET-B receptor antagonism attenuated the increase from baseline in SVR (7 ± 1 vs. 28 ± 7 RU, P < 0.05) and PVR (0.7 ± 0.2 vs. 3.2 ± 0.7 RU, P < 0.05) and decrease from baseline in CO (−0.9 ± 0.1 vs. −1.7 ± 0.3 l/min, P < 0.05) and SVO2 (−7 ± 3 vs. −20 ± 3%, P < 0.05) observed with S6c alone. In summary, this study demonstrates an important role of ET-B receptor activation in vivo, which results in increases in plasma ET-1 and systemic and pulmonary vasoconstriction and reductions in CO and SVO2. This study also supports a modest role for the ET-A receptor in mediating the systemic vasoconstrictor response to high-dose S6c.

endothelin; sarafotoxin S6c; peptide

ENDOTHELIN (ET)-1 is a 21-amino acid, potent vasoconstricting peptide isolated from endothelial cells of porcine aorta (19, 32). ET-1 is a member of a family of endothelin peptides that also includes ET-2 and ET-3 (10). ET-1 is the predominant endothelin-derived vasoconstricting peptide in humans. ET-1 is released from endothelial cells under normal physiological conditions through a constitutive pathway, resulting in basal concentrations of circulating and tissue ET-1 immuno-reactivity. Circulating ET-1 has been shown to be elevated and may play an important role in cardiovascular disease states such as congestive heart failure (CHF), atherosclerosis, and myocardial infarction (14, 20, 30). ET-1 mediates its effects through at least two receptor subtypes, ET-A and ET-B (9, 23).

The ET-A receptor is expressed on vascular smooth muscle cells and has a high affinity for ET-1 compared with ET-3 (9, 31). The ET-A receptor activation results in vasoconstriction via activation of a phospholipase C-mediated increase in smooth muscle cell intracellular calcium (1, 18). The ET-B receptor was initially thought to be expressed exclusively on vascular endothelial cells and mediate the production of the vasodilating substances nitric oxide (NO) and prostacyclin [prostaglandin I2 (PGI2)] (8, 26). More recent studies have demonstrated expression of the ET-B receptor on vascular smooth muscle cells from the aorta and pulmonary and coronary arteries.

In addition to mediating vasorelaxation, a vasoconstrictor role for the ET-B receptor has been demonstrated in vitro, and studies suggest that ET-B receptor blockade may be necessary to reverse all of the vasoconstrictor actions of ET-1 (5, 7, 12, 27). Specifically, ET-A receptor antagonism with the selective ET-A receptor antagonist BQ-123 has been shown in vitro to incompletely inhibit the actions of ET-1 (21, 28). Furthermore, Fukuroda et al. (6) demonstrated in vitro that the combination of a selective ET-B receptor antagonist, BQ-788, with BQ-123 produced a synergistic inhibition of ET-1-induced vasoconstriction. In vivo, Leadly et al. (13) demonstrated in normal dogs a mild vasconstrictor response to low doses of the selective ET-B agonist sarafotoxin S6c (S6c) with increases in peripheral but not renal vascular resistance. In contrast, Haynes et al. (7) reported that selective ET-B receptor activation with S6c results in significant vasoconstriction in the human forearm.

Although the ET-B receptor may have direct vasoconstricting actions in isolated vessels, it may also possess the ability to indirectly cause vasoconstriction by increasing endogenous ET-1 secretion, resulting in ET-A receptor activation. Specifically, in vitro studies demonstrate that the ET-B receptor upregulates the preproET-1 gene with increased ET-1 secretion from cultured endothelial and mesangial cells and cardiac myocytes (11, 25, 29, 33). Such an action could therefore represent an additional mechanism, via local activation of the ET-A receptor, of ET-B receptor-mediated vasoconstriction. The in vivo receptor mechanisms by which ET-B agonists exert hemodynamic effects remain unclear.
The objective of the current study was to extend previous studies and define in vivo the actions of ET-B receptor stimulation employing the selective ET-B agonist S6c on regional vascular resistances, cardiac output (CO), and mixed venous oxygen saturation (SvO₂). In addition, the receptor mechanisms by which S6c exerts its hemodynamic effects were investigated. ET-B activation with S6c was performed in two additional groups in the presence of a selective ET-A receptor antagonist, FR-139317, or of a mixed ET-A/ET-B receptor antagonist, SB-209670. We tested the hypothesis that activation of the ET-B receptor in vivo results in systemic and pulmonary but not renal or coronary vasoconstriction in association with reductions in CO. Furthermore, we tested the hypothesis that infusion of S6c is associated with increased circulating ET-1, which may contribute indirectly, via activation of the ET-A receptor, to the observed hemodynamic effects of ET-B receptor stimulation.

METHODS

Experiments were performed in four groups of normal dogs weighing between 17 and 21 kg as follows. Group 1 (n = 5) served as a time control group and underwent the same surgical preparation and investigations as the study groups. Group 2 (n = 5) underwent infusion of the selective ET-B receptor agonist S6c (Sigma Chemical, St. Louis, MO) at 5, 25, and 50 ng·kg⁻¹·min⁻¹ alone. Group 3 (n = 6) underwent infusion of S6c with a continuous infusion of the ET-A receptor antagonist FR-139317 (Abbott Laboratories, Chicago, IL) at 10 μg·kg⁻¹·min⁻¹. Group 4 (n = 4) underwent infusion of S6c with a continuous infusion of the mixed ET-A/ET-B receptor antagonist SB-209670 (SmithKline Beecham, King of Prussia, PA) at 40 μg·kg⁻¹·min⁻¹. All studies were approved by the Institutional Animal Care and Use Committee.

Table 1. Baseline cardiovascular hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (Control)</th>
<th>Group 2 (S6c Alone)</th>
<th>Group 3 (S6c + FR-139317)</th>
<th>Group 4 (S6c + SB-209670)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>124 ± 4</td>
<td>90 ± 5</td>
<td>106 ± 8</td>
<td>95 ± 6</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>2.8 ± 0.2</td>
<td>2.9 ± 0.3</td>
<td>3.0 ± 0.2</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td>RAP, mmHg</td>
<td>1.1 ± 1</td>
<td>2.4 ± 0.8</td>
<td>2.3 ± 0.8</td>
<td>3 ± 0.5</td>
</tr>
<tr>
<td>PCWP, mmHg</td>
<td>2.0 ± 0.4</td>
<td>4.6 ± 1.1</td>
<td>3.8 ± 0.7</td>
<td>3.1 ± 0.8</td>
</tr>
<tr>
<td>PAP, mmHg</td>
<td>12 ± 1</td>
<td>15 ± 2</td>
<td>15 ± 1</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>CBF, ml/min</td>
<td>59 ± 5</td>
<td>54 ± 16</td>
<td>54 ± 11</td>
<td>49 ± 7</td>
</tr>
<tr>
<td>RBF, ml/min</td>
<td>251 ± 34</td>
<td>242 ± 35</td>
<td>211 ± 20</td>
<td>234 ± 39</td>
</tr>
<tr>
<td>SVR, mmHg·l⁻¹·min⁻¹</td>
<td>42 ± 5</td>
<td>30.3 ± 2.6</td>
<td>36 ± 5</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>PVR, mmHg·l⁻¹·min⁻¹</td>
<td>3.8 ± 0.4</td>
<td>3.6 ± 0.4</td>
<td>3.8 ± 0.4</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>CVR, mmHg·ml⁻¹·min⁻¹</td>
<td>1.9 ± 0.3</td>
<td>2.8 ± 1.2</td>
<td>2.1 ± 0.6</td>
<td>2.0 ± 0.4</td>
</tr>
<tr>
<td>RVR, mmHg·ml⁻¹·min⁻¹</td>
<td>0.49 ± 0.08</td>
<td>0.36 ± 0.02</td>
<td>0.52 ± 0.07</td>
<td>0.44 ± 0.09</td>
</tr>
</tbody>
</table>

Values are means ± SE. S6c, sarafotoxin S6c; MAP, mean arterial pressure; CO, cardiac output; RAP, right atrial pressure; PCWP, pulmonary capillary wedge pressure; PAP, pulmonary arterial pressure; CBF, coronary blood flow; RBF, renal blood flow; SVR, systemic vascular resistance; PVR, pulmonary vascular resistance; CVR, coronary vascular resistance; RVR, renal vascular resistance.

Dogs were maintained on a normal sodium diet with standard dog chow (Lab Canine Diet 5006; Purina Mills, St. Louis, MO) with free access to water. All dogs were fasted the night before the acute experiment and allowed access only to water. On the day of the acute experiment, the dogs were anesthetized with intravenous pentobarbital sodium (30 mg/kg), with supplemental doses given throughout the experiment as needed. The dogs were intubated and ventilated (Harvard Apparatus, South Natick, MA) on room air supplemented with oxygen at 41/min.

The right external jugular vein was exposed, and an oxymetric flow-directed, thermodilution pulmonary artery catheter was placed (model P7110, Abbott Critical Care Systems, Chicago, IL). The right femoral vein was cannulated with polyethylene catheter for the infusion of S6c, saline, and other compounds. The right femoral artery was also cannulated for the measurement of mean arterial pressure (MAP). A left flank incision was made, and the left renal artery was exposed. An electromagnetic flow probe was placed on the left renal artery and connected to a flowmeter (model FM 5010; Carolina Medical Electronics, King, NC). A left thoracotomy was made through the fourth intercostal space, exposing the heart and the pericardium. The left lung was gently retracted and a pericardiotomy was made, exposing the left ventricle and the left circumflex artery. After minimal dissection, an electromagnetic flow probe was placed on the left circumflex artery and connected to a flowmeter (model FM 5010, Carolina Medical Electronics). Renal blood flow (RBF), coronary...
blood flow (CBF), MAP, and filling pressures were recorded on a Gould model 2200 strip recorder (Gould Electronics, Cleveland, OH).

After completion of the surgery, the dog was allowed to equilibrate for 1 h with infusion of saline at 1 ml/min, which continued throughout the protocol. After the 1-h equilibration period, a 15-min baseline period for measurement of hemodynamics was undertaken. The hemodynamic measurements included MAP, pulmonary capillary wedge pressure (PCWP), SVO₂, pulmonary artery pressure (PAP), right atrial pressure (RAP), RBF, CBF, and CO in triplicate by thermodilution method (model 93–121A, 7F; American Edwards Laboratories, Santa Ana, CA). Systemic (SVR), pulmonary (PVR), coronary (CVR) and renal (RVR) vascular resistances were calculated utilizing the following formulas: 

\[
SVR = \frac{(MAP - RAP) \times CO}{\text{MAP} - \text{RAP}} \\
PVR = \frac{(PAP - PCWP) \times CO}{\text{PAP} - \text{PCWP}} \\
CVR = \frac{(MAP - RAP) \times CBF}{\text{MAP} - \text{RAP}} \\
RVR = \frac{(MAP - RAP) \times RBF}{\text{MAP} - \text{RAP}} 
\]

Group 1 underwent only continuous infusion of saline with hemodynamic measurements as mentioned above. In Group 2, after the equilibration and baseline period, S6c was infused at 5, 25, and 50 ng·kg⁻¹·min⁻¹, with each period lasting 30 min. Infusion of S6c at 5 ng·kg⁻¹·min⁻¹ was begun with a 15-min lead-in period followed by a 15-min clearance during which data was collected. Infusion dose was increased to 25 ng·kg⁻¹·min⁻¹, with a 15-min lead-in period and followed by a 15-min clearance. Then, the infusion dose was increased to 50 ng·kg⁻¹·min⁻¹, with a 15-min lead-in period and followed by a 15-min clearance. Data collection and hemodynamic measurements were undertaken at the end of each period. After the last S6c period, infusion of S6c was stopped. A 40-min washout period followed with measurements of hemodynamic parameters.

Group 3 underwent a continuous infusion of FR-139317 (10 µg·kg⁻¹·min⁻¹) in addition to S6c. FR-139317 was started after the equilibration period and was continued throughout S6c infusions. The dose of FR-139317 was based on previous studies by the investigators, which have demonstrated effective antagonism of endogenous and exogenous ET-1 (4). After 30 min of FR-139317, hemodynamic measurements were assessed. S6c was infused at 5, 25, and 50 ng·kg⁻¹·min⁻¹ in the same manner as group 2. After this period, infusions of S6c and FR-139317 were stopped. A 40-min washout period was followed by measurements of hemodynamic parameters.

Instead of FR-139317, group 4 underwent a continuous infusion of SB-209670 (40 µg·kg⁻¹·min⁻¹) in addition to an infusion of S6c.

Blood for ET-1 analysis was collected into EDTA tubes, immediately placed on ice, and centrifuged at 2,500 revolutions/min at 4°C. Plasma was separated and stored at −20°C until the assay. Plasma levels of ET-1 were determined using specific radioimmunoassays as previously described (17).

Data from each period were averaged and expressed as means ± SE. Within each group, repeated measures were analyzed by analysis of variance (ANOVA) followed by Fisher's least-significant difference test when appropriate. For groups 1, 2, 3, and 4, absolute changes from baseline between groups were analyzed with ANOVA and an unpaired Student’s t-test. Statistical significance was accepted at a value of P < 0.05.

**RESULTS**

ET-B activation with S6c vs. time control. Table 1 reports baseline hemodynamic data in each group. MAP in group 1 was statistically higher compared with group 2, and RAP was statistically lower in group 1 than in group 3. Baseline PCWP was lower in group 1 compared with group 2. There was no statistical difference between the remaining baseline values in the four
groups. S6c infusion decreased MAP from baseline during 25 ng·kg\(^{-1}\)·min\(^{-1}\) (81.6 ± 7 vs. 90.6 ± 5 mmHg, \(P < 0.05\)) and 50 ng·kg\(^{-1}\)·min\(^{-1}\) (81.8 ± 8 vs. 90.6 ± 5 mmHg, \(P < 0.05\)), which returned to baseline during recovery. Furthermore, as illustrated in Fig. 1, the absolute change in MAP from baseline was different between group 1 (time control) and group 2 (S6c) at 5 ng·kg\(^{-1}\)·min\(^{-1}\) (−2.6 ± 2.1 vs. 3.8 ± 2.5 mmHg, \(P < 0.05\)), 25 ng·kg\(^{-1}\)·min\(^{-1}\) (−9.0 ± 2.2 vs. 8.3 ± 3.8 mmHg, \(P < 0.05\)), and 50 ng·kg\(^{-1}\)·min\(^{-1}\) (−8.6 ± 5 vs. 4.8 ± 6 mmHg, \(P < 0.05\)). There was no change in RAP, PAP, CBF, or CVR in either group 1 or group 2. A modest increase in PCWP in the control group was observed (1.7 ± 0.3 vs. 2.2 ± 0.6 mmHg, \(P < 0.05\)), which was not observed in group 2 with S6c. RBF decreased in both group 1 and group 2 together with increases in RVR. There was no difference in the absolute change from baseline between groups with regard to RBF or RVR.

Figure 1 also illustrates the absolute change in CO from baseline in time control and S6c during vehicle and S6c infusion. CO decreased with S6c compared with the time-control group during the 50 ng·kg\(^{-1}\)·min\(^{-1}\) infusion (−1.7 ± 0.4 vs. −0.5 ± 0.1 l/min, \(P < 0.05\)) and returned to baseline during recovery. Figure 2 illustrates the absolute change from baseline in SVR in the two groups. SVR increased with S6c compared with time control during 50 ng·kg\(^{-1}\)·min\(^{-1}\) infusion (3.2 ± 0.7 vs. 0.9 ± 0.3 RU, \(P < 0.05\)) and returned to control during recovery. Figure 2 also illustrates the absolute change from baseline in PVR between the two groups. PVR increased compared with time control during the 50 ng·kg\(^{-1}\)·min\(^{-1}\) infusion (28 ± 7 vs. 14 ± 3 resistance units (RU), \(P < 0.05\)) and returned to control during recovery.

Figure 3 illustrates circulating ET-1 in each of the dogs in group 2 before and during S6c infusion of 50 ng·kg\(^{-1}\)·min\(^{-1}\). Plasma ET-1 increased (14.9 ± 1.3 vs. 19.9 ± 2.6 pg/ml, \(P < 0.05\)) in group 2 during the 50 ng·kg\(^{-1}\)·min\(^{-1}\) infusion of S6c.

S6c with selective ET-A (FR-139317) vs. ET-A/ET-B (SB-209670) receptor blockade. No difference was observed between group 2 with S6c alone and group 3 with S6c and FR-139317 in any of the hemodynamic parameters except PCWP. Specifically, a modest but greater decrease was observed from baseline in PCWP in group 3 during the 25 ng·kg\(^{-1}\)·min\(^{-1}\) (−1.5 ± 0.4 vs. −0.3 ± 0.4 mmHg, \(P < 0.05\)) and 50 ng·kg\(^{-1}\)·min\(^{-1}\) (−1.6 ± 0.5 vs. −0.4 ± 0.2 mmHg, \(P < 0.05\)) administration of S6c compared with group 2 with S6c alone.
DISCUSSION

The current investigation was designed to define the in vivo cardiovascular actions of ET-B receptor activation on regional vascular resistances, CO, MAP, SvO₂, a measure of effective tissue perfusion, and circulating ET-1 concentrations. In addition, this study specifically investigated the ET-1 receptors through which S6c exerts its cardiovascular effects. The current study demonstrates that activation of the ET-1 receptor in vivo with high concentrations of S6c results in marked cardiovascular responses, which include systemic and pulmonary vasoconstriction, reductions in CO and MAP, and decreases in SvO₂, in association with increases in circulating ET-1. Furthermore, this study supports a dual mechanism by which S6c mediates its cardiovascular actions. In response to high-dose S6c, ET-A receptor antagonism blocked any further increase in SVR (Table 2). Nonetheless, the addition of an ET-B receptor antagonist resulted in a greater attenuation in S6c-mediated increases in both SVR and PVR (Fig. 6), underscoring the predominant role for the ET-B receptor in mediating the vasoconstrictor actions of the ET-B agonist S6c.

Controversy persists with regard to the vascular actions of the ET-B receptor, which has demonstrated a vasodilatory role secondary to release of PGI₂ or NO (8, 25). However, other studies support an arterial vasoconstrictor role at least in some isolated arterial preparations (20, 27). In the current study, we investigated the regional vascular responses in vivo to low and high doses of S6c. At none of the concentrations employed

Table 2. Cardiovascular hemodynamics during S6c and S6c + FR-139317 administration

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>5 ng·kg⁻¹·min⁻¹</th>
<th>25 ng·kg⁻¹·min⁻¹</th>
<th>50 ng·kg⁻¹·min⁻¹</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1: S6c</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MAP, mmHg</td>
<td>90 ± 5</td>
<td>87 ± 5</td>
<td>81 ± 7</td>
<td>81 ± 8</td>
</tr>
<tr>
<td></td>
<td>HR, beats/min</td>
<td>118 ± 6</td>
<td>122 ± 6</td>
<td>123 ± 5</td>
<td>117 ± 11</td>
</tr>
<tr>
<td></td>
<td>CO, I/min</td>
<td>2.9 ± 0.3</td>
<td>2.9 ± 0.3</td>
<td>2.2 ± 0.2</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>RAP, mmHg</td>
<td>2.4 ± 0.8</td>
<td>2.3 ± 0.9</td>
<td>2.8 ± 1.0</td>
<td>2.1 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>PCWP, mmHg</td>
<td>4.6 ± 1.1</td>
<td>4.4 ± 1.0</td>
<td>4.3 ± 1.0</td>
<td>4.2 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>PAP, mmHg</td>
<td>15 ± 2</td>
<td>15 ± 2</td>
<td>14 ± 3</td>
<td>14 ± 3</td>
</tr>
<tr>
<td></td>
<td>CBF, ml/min</td>
<td>54 ± 16</td>
<td>61 ± 11</td>
<td>51 ± 11</td>
<td>54 ± 15</td>
</tr>
<tr>
<td></td>
<td>RBF, ml/min</td>
<td>242 ± 35</td>
<td>246 ± 24</td>
<td>194 ± 22</td>
<td>168 ± 23</td>
</tr>
<tr>
<td></td>
<td>SVR, mmHg·l⁻¹·min⁻¹</td>
<td>30.3 ± 2.6</td>
<td>29.7 ± 3</td>
<td>35.9 ± 3</td>
<td>56.3 ± 5.6</td>
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<tr>
<td></td>
<td>PVR, mmHg·l⁻¹·min⁻¹</td>
<td>36.0 ± 4</td>
<td>38.0 ± 5</td>
<td>4.7 ± 6</td>
<td>6.8 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>CBF, ml·min⁻¹</td>
<td>2.8 ± 1.2</td>
<td>1.7 ± 0.4</td>
<td>1.8 ± 0.3</td>
<td>2.1 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>RBF, ml·min⁻¹</td>
<td>0.36 ± 0.02</td>
<td>0.36 ± 0.03</td>
<td>0.41 ± 0.02</td>
<td>0.5 ± 0.08</td>
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</table>

Values are means ± SE. HR, heart rate. *P < 0.05 vs. baseline; **P < 0.05 vs. 5 ng·kg⁻¹·min⁻¹; ***P < 0.05 vs. 25 ng·kg⁻¹·min⁻¹; ****P < 0.05 vs. 50 ng·kg⁻¹·min⁻¹; *****P < 0.05 vs. group 1.
was a vasodilatory response observed compared with the time-control group. Although this may suggest that in the anesthetized dog a vasodilatory component does not exist, the observation may suggest that the release of PGI₂ and/or NO offsets the direct vasoconstricting actions of the ET-B receptor. Indeed, in previous studies, we have reported that the vasoconstricting actions of ET-1 are exaggerated vasoconstrictor responses in the presence of pharmacological inhibition of NO generation (15).

Although there was no observed vasodilatory response to S6c, a marked vasoconstrictor response, compared with time control, was observed in the systemic and pulmonary circulations in response to high concentrations of the ET-B agonist. This observation is consistent with investigations in isolated arterial resistance vessels as well as in isolated pulmonary arteries that ET-B receptors may be involved with peripheral as well as pulmonary vasoconstriction (6, 12). Two vascular beds that were not responsive to S6c were the coronary and renal circulations in which CVR did not change with ET-B activation and RVR increased but to a degree not different from the time control. Thus the current study confirms reports of a vasoconstrictor but not vasodilatory role for the ET-B receptor in the dog and extends previous reports to establish a heterogeneous response in the systemic and pulmonary circulations but not in the coronary or renal circulations.

A marked decrease in CO and MAP occurred in response to S6c compared with the time control. The mechanism of this decrease is likely multifactorial and includes increases in cardiac afterload secondary to the increase in SVR, venoconstriction with a reduction in venous return and cardiac preload, and a possible direct negative inotropic response. The lack of change in CBF and CVR suggests that ischemia did not contribute to the decrease in CO. The decrease in SvO₂ during S6c also suggests that the reduction in CO together with vasoconstriction had physiological significance because this parameter decreased significantly with ET-B stimulation and returned to baseline during S6c washout.

The current investigation extends previous studies and investigates ET-B activation in vivo in the presence and absence of ET-B receptor antagonism with a dual ET-A and ET-B receptor antagonist, SB-209670. SB-209670 is a potent nonpeptide antagonist at both the ET-A and ET-B receptors. Thus, in the cloned human ET-A and ET-B receptors, SB-209670 can inhibit ET-1 binding with Kᵢ values of 0.2 and 18 nM, respectively (22). SB-209670 produces parallel rightward shifts in the concentration-response curve for ET-1 in isolated rat aorta (24). At higher concentrations, SB-209670 can competitively antagonize ET-B receptors by producing parallel rightward shifts in the response to ET-1 in isolated rabbit pulmonary artery (24). In the dog, SB-209670 blocks ET-A receptor-mediated vasoconstriction (2).

These in vivo studies confirm and extend in vitro observations identifying an important role for the ET-B
receptor in mediating the cardiovascular actions of S6c as SB-290670 attenuated systemic and pulmonary vasoconstriction. Specifically, in contrast to selective ET-A receptor antagonism alone, dual inhibition of ET-A and ET-B receptors with SB-209670 significantly attenuated the biological response to S6c, namely, the increase in SVR and PVR (Fig. 6). Furthermore, the reduction in CO was attenuated by dual ET-A and ET-B receptor blockade. The decrease in SVR with S6c was completely abolished only by dual ET receptor antagonism. This study supports the conclusion that at pharmacological concentrations, S6c, via the ET-B receptor, has potent cardiovascular properties.

It should be noted that the cardiovascular responses to S6c administration in the presence of ET-A receptor antagonism with FR-139317 was not different from S6c alone, with the exception that ET-A receptor antagonism abolished the increase in SVR to high-dose S6c (Table 2). This suggests that, perhaps locally, the ET-B receptor in the control of systemic vascular tone may act indirectly to activate the ET-A receptor. This explanation is consistent with in vitro studies that report that ET-B receptor activation may increase ET-1 production and secretion (11, 25, 29, 33). Nonetheless, the general lack of attenuation of the cardiovascular responses to S6c with FR-139317, as one would expect, further supports the conclusion that the actions of S6c were principally secondary to ET-B receptor stimulation.

A limitation of the current study is that the investigation was performed in anesthetized animals. We therefore cannot exclude that anesthesia may have had a modulating action.

In summary, this study demonstrates an important cardiovascular role for ET-B receptor activation at pharmacological concentrations in mediating systemic and pulmonary vasoconstriction and reductions in CO and SVR. Although selective ET-A receptor blockade attenuated the systemic vasoconstrictor action of high-dose S6c, we provide evidence that dual ET-A/ET-B receptor antagonism attenuates these potent cardiovascular actions of the ET-B receptor. Such cardiopulmonary protective properties of ET-B receptor blockade may represent an important therapeutic strategy in states of ET-1 activation.

Perspectives

In the current investigation, cardiovascular responses were present only at pharmacological concentrations of S6c. Nonetheless, this may have pathological relevance in cardiovascular disease states such as CHF, shock, pulmonary hypertension, and atherosclerosis (14, 20, 30). Specifically, in states where ET-1 production is increased or the expression or sensitivity of the ET-B receptor is enhanced or the release of endogenous vasodilators is attenuated, the ET-B receptor may mediate vasoconstrictor responses not observed under physiological conditions. Indeed, in recent studies by Cannon et al. (3) in experimental CHF, a vasoconstrictor response to sarafotoxin was observed in the coronary circulation that was not noted in the normal dog. Additionally, in human CHF Love et al. (16) have demonstrated that ET-B-mediated forearm vasoconstriction by S6c is enhanced. Thus the current investigation has both pathophysiological and therapeutic relevance in supporting a role for ET-B as well as ET-A receptor blockade in the disease states of elevated plasma ET-1.

We thank Lawrence L. Aarhus for his expert surgical assistance and Denise M. Heublein and Sharon M. Sandberg for their bioassay expertise. We acknowledge the generosity of Dr. T. J. Opgenorth of Abbott Laboratories for providing the compound FR-139317.

Funding for this project was provided by the National Kidney Foundation of the Upper Midwest, the Mayo Foundation, National Heart, Lung, and Blood Institute Grant HL-34463, a grant from the Miami Heart Research Institute, and the Bruce and Ruth Rapaport Program in Vascular Biology.

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Received 18 January 1996; accepted in final form 25 September 1997.

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