Endothelin-dependent effects limit flow-induced dilation of conductance coronary vessels after blockade of nitric oxide formation in conscious dogs

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Abstract

Objective: To determine whether endothelin (ET)-dependent effects limit shear stress-induced dilation of large epicardial coronary arteries after blockade of nitric oxide (NO) formation. Methods: In conscious dogs instrumented for measuring coronary blood flow (CBF) and external diameter (CD) of the circumflex coronary artery, flow-dependent CD dilation was elicited by intracoronary (ic) adenosine (500 ng kg⁻¹ min⁻¹). Results: Ic adenosine increased CBF by 28±4 from 38±5 ml min⁻¹ and CD by 0.25±0.03 from 3.53±0.07 mm without other hemodynamic effects. After N⁴-nitro-L-arginine methyl ester (L-NAME), baseline CD fell (P<0.01) to 3.35±0.08 mm but CBF was not significantly altered (36±5 ml min⁻¹). CBF increases caused by adenosine were smaller (17±2 ml min⁻¹, P<0.05) and CD responses were nearly abolished (0.02±0.01 mm, P<0.01). Ic Ro 61-1790, an ET₄ receptor blocker, given after L-NAME did not significantly influence baseline CBF (36±5 ml min⁻¹) but CD responses (0.10±0.01 mm) were partially restored (P<0.01). In contrast, blockade of ET₄ receptors with Ro 46-8443 after L-NAME had no further effects on CD and CBF responses to adenosine. Conclusion: ET₄ receptor-mediated effects limit flow-dependent dilation of large epicardial coronary arteries in conscious dogs. Suppression of the L-arginine/NO-dependent pathway with L-NAME reveals significant ET-dependent effects.

Keywords: Adenosine; Coronary circulation; Endothelial function; Endothelins; Nitric oxide

1. Introduction

Shear stress is one of the major determinants of arterial tone of large and small coronary vessels. Acting as an upstream signalling mechanism, shear stress contributes to coordinate serial segmental vascular responses within the coronary bed [1]. Nitric oxide (NO) formation by the endothelium is pivotal in this process. Blockade of NO formation prevents flow-induced dilation in large [2–4] and small [5] coronary arteries.

Several studies have now reported that shear stress applied to endothelial cells in culture influenced endothelin (ET) activity. Changes in ET mRNA and in ET release have been associated with altered shear stress levels in vitro [6–13]. This raises the possibility that ET may play a role in flow-dependent responses in vivo. In isolated pressurized mesenteric arteries of spontaneously hypertensive rats, chronic blockade of ET₄ receptors has been reported to improve flow-dependent dilation [14]. The functional significance of these observations has not been addressed in vivo.

Because agents triggering NO formation or increasing
cGMP formation impaired ET-dependent responses and prevented stimulated ET release in vitro [15–17], we hypothesized that NO may normally suppress an ET-dependent component triggered by elevated shear stress levels in vivo. Conversely, a contribution of ET during increases in shear stress may become apparent after blockade of NO formation.

Our primary objective was therefore to determine whether ET-dependent tone influenced flow-dependent dilation of large epicardial coronary arteries in conscious dogs with normal and impaired NO formation. Using selective ET<sub>A</sub> and ET<sub>B</sub> receptor blockers, we further examined which ET receptor subtype was involved.

2. Methods

2.1. Instrumentation

Under general anesthesia with sodium pentobarbital (30 mg kg<sup>−1</sup>, intravenous [iv]), artificial ventilation and sterile conditions, nine mongrel dogs (31±1 kg) underwent a left thoracotomy at the fifth intercostal space and were instrumented as previously described [4]. Briefly, the proximal circumflex coronary artery was instrumented with a catheter for drug delivery, a Doppler flow probe for monitoring coronary blood flow (CBF) velocity and with miniature ultrasonic piezoelectric crystals sutured on opposite sides of the vessels for measuring external coronary artery diameter (CD) with a Sonomicrometer (model 120.2, Triton, Pasadena, CA, USA). A calibration factor (ml min<sup>−1</sup> kHz<sup>−1</sup>) to calculate CBF (in ml min<sup>−1</sup>) was obtained from the vessel cross-sectional area under the probe measured at necropsy and flow velocity measurements.

2.2. Protocols

Experiments were initiated 2 to 4 weeks after surgery in conscious healthy dogs pretreated with iv indomethacin (5.0 mg kg<sup>−1</sup>). A bolus of 50 ng kg<sup>−1</sup> nitroglycerin (Parke-Davis, Scarborough, Ontario, Canada) was given intracoronary (ic) to evaluate the reactivity of large epicardial coronary arteries which had to be >0.20 mm to be considered adequate.

Ic infusions of 200 and 500 ng kg<sup>−1</sup> min<sup>−1</sup> adenosine (Sigma, St. Louis, MO, USA) were performed until a steady-state was reached, i.e., 4–6 min after the beginning of the infusion. These doses of adenosine were selected on the basis of our earlier studies showing that CD responses to adenosine were flow-dependent and sensitive to the blockade of NO formation with ic N<sup>ω</sup>-nitro-l-arginine methyl ester (l-NAME; 50.0 μg kg<sup>−1</sup> min<sup>−1</sup> for 12 min, Sigma) [4,18].

2.2.1. Blockade of NO formation and ET<sub>A</sub> receptors

Adenosine infusions were performed in nine dogs before l-NAME, after l-NAME with and without ic 5-methylpyridine-2-sulfonic acid 6-(2-hydroxyethoxy)-5-(2-methoxyphenox)-2-(2-1H-tetrazol-5-yl-pyrind-4-yl)-pyrimidin-4-ylamide disodium salt (Ro 61-1790; 2.5 μg kg<sup>−1</sup> min<sup>−1</sup>×10 min+0.25 μg kg<sup>−1</sup> min<sup>−1</sup>×20 min, Hoffmann-La Roche Basel, Switzerland), a selective ET<sub>A</sub> receptor blocker [19]. In an earlier study, this dose of Ro 61-1790 reduced (P<0.05) the fall in CBF caused by an ic bolus of ET-1 (0.1 μg) from 21±2 to 7±2%. [20]. In vitro, Ro 61-1790 (10<sup>−6</sup> mol l<sup>−1</sup>) abolished ET-1 induced contractions but did not alter sarafotoxin 6c-induced contractions (3·10<sup>−6</sup> mol l<sup>−1</sup>) [21]. On a different day, the effects of ic Ro 61-1790 alone (without l-NAME) on adenosine-induced responses were examined in eight dogs.

2.2.2. Blockade of NO formation and ET<sub>B</sub> receptors

Adenosine infusions were performed in seven dogs before l-NAME, after ic l-NAME (50.0 μg kg<sup>−1</sup> min<sup>−1</sup> for 12 min) with and without ic (5)-4-tert.-butyl-N-[6-(2,3-dihydroxypropoxy)-5-(2-methoxyphenox)-2-(4-methoxyphenyl)pyrimidin-4-yl]benzenesulfonamide (Ro 46-8443; 30.0 μg kg<sup>−1</sup> min<sup>−1</sup>×10 min+1.0 μg kg<sup>−1</sup> min<sup>−1</sup> thereafter, Hoffmann-La Roche), a selective ET<sub>B</sub> receptor blocker [22]. In an earlier study [20], this dose of Ro 46-8443 was adequate to prevent (P<0.05) the early rise (63±17 to 2±2%) and the late decrease (24±3 to 6±2%) in CBF caused by an ic bolus of sarafotoxin 6c (0.3 μg), a selective ET<sub>B</sub> receptor agonist [23]. In vitro, Ro 46-8443 (10<sup>−6</sup> mol l<sup>−1</sup>) had no effects on ET-1 induced contractions but Ro 46-8443 (10<sup>−7</sup> mol l<sup>−1</sup>) abolished sarafotoxin 6c-induced contractions (3·10<sup>−6</sup> mol l<sup>−1</sup>) [21]. On a different day, the effects of ic Ro 46-8443 alone (without l-NAME) on adenosine-induced responses was examined in five dogs.

2.3. Data analysis

Data are reported as mean±SEM. Paired t-tests were used to compare left ventricular pressure (LVP), the first derivative of LVP over time (LV dP/dt), mean arterial pressure (MAP), heart rate (HR), CD and CBF responses to adenosine to baseline values under the various experimental conditions [24].

Simultaneous comparisons of baselines or responses to graded doses of adenosine before and after l-NAME with and without Ro 61-1790 or Ro 46-8443 were performed with analysis of variance (ANOVA) for repeated measurements [25] to assess overall statistical significance. For any given dose of adenosine, comparisons of responses were performed with ANOVA followed by the Bonferroni’s t-tests to isolate specific contrasts. Paired t-tests were used to compare responses to a given dose of adenosine before and after Ro 61-1790 or Ro 46-8443 alone. All experimen-
3. Results

Except for significant increases in CBF and CD, ic adenosine had no other hemodynamic effects under the various experimental conditions.

3.1. Combined blockade of NO formation and $E_{TA}$ receptors

Baseline hemodynamics before $\text{l-NAME}$, after $\text{l-NAME}$ and after $\text{l-NAME+Ro 61-1790}$ are reported in Table 1. $\text{l-NAME}$ significantly increased LVP ($P<0.01$) and MAP ($P<0.01$) and decreased HR ($P<0.01$) and CD ($P<0.01$). CBF and LV $dP/dt$ were not altered. Ro 61-1790 given after $\text{l-NAME}$ decreased ($P<0.01$) LVP and MAP and increased ($P<0.01$) baseline CD without significantly altering CBF, LV $dP/dt$ and HR.

CBF and CD responses to adenosine 200 and 500 are summarized in Fig. 1. Before $\text{l-NAME}$, adenosine 200 and 500 increased ($P<0.01$) CD and CBF. After $\text{l-NAME}$, CD responses to adenosine 200 and 500 were dramatically reduced ($P<0.01$) in spite of the limited effects on CBF responses. After Ro 61-1790 (given after $\text{l-NAME}$), CD responses to adenosine 200 and 500 were augmented.

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Table 1

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>l-NAME</th>
<th>l-NAME + Ro 61-1790</th>
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<tbody>
<tr>
<td>$n$=9</td>
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<tr>
<td>Left ventricular pressure</td>
<td>115±2</td>
<td>125±3*</td>
<td>114±3*</td>
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<tr>
<td>(mmHg)</td>
<td></td>
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</tr>
<tr>
<td>LV $dP/dt$</td>
<td>2630±90</td>
<td>2579±91</td>
<td>2673±113</td>
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<tr>
<td>(mmHg s$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td>94±1</td>
<td>104±2*</td>
<td>91±2*</td>
</tr>
<tr>
<td>(mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate</td>
<td>73±2</td>
<td>55±2*</td>
<td>61±2</td>
</tr>
<tr>
<td>(beats min$^{-1}$)</td>
<td></td>
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<tr>
<td>Coronary blood flow</td>
<td>37±3</td>
<td>36±3</td>
<td>36±4</td>
</tr>
<tr>
<td>(ml min$^{-1}$)</td>
<td></td>
<td></td>
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<tr>
<td>Coronary diameter</td>
<td>3.53±0.05</td>
<td>3.34±0.05*</td>
<td>3.45±0.06*</td>
</tr>
<tr>
<td>(mm)</td>
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</table>

* $P<0.01$ vs. previous treatment.

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Fig. 1. Changes in external coronary diameter (CD, top panels) and coronary blood flow (CBF, bottom panels) caused by ic infusions of adenosine 200 and 500 ng kg$^{-1}$ min$^{-1}$ before $N^\text{-nitro-}$arginine methyl ester ($\text{l-NAME}$), after ic $\text{l-NAME}$ and after $\text{l-NAME+Ro 61-1790}$, a selective $E_{TA}$ receptor blocker. Ro 61-1790 partially reversed the inhibitory effects of $\text{l-NAME}$ on adenosine-induced responses. * $P<0.05$ vs. previous treatment; † $P<0.01$ vs. previous treatment ($n=9$).
(P<0.01) as compared to responses after l-NAME alone but CBF responses were not significantly altered.

3.2. Blockade of ET<sub>α</sub> receptors

Ro 61-1790 alone increased (P<0.01) baseline CD from 3.51±0.05 to 3.65±0.06 mm and decreased (P<0.01) MAP from 95±2 to 89±2 mmHg. LVP, LV dP/dt, HR and CBF were not significantly altered.

CD and CBF increases (P<0.01) caused by adenosine 200 were not statistically different before and after Ro 61-1790 (Fig. 2). CD increases caused by adenosine 500 were slightly decreased (P<0.01) but CBF responses were maintained.

3.3. Combined blockade of NO formation and ET<sub>B</sub> receptors

The pattern of hemodynamic effects caused by l-NAME was similar to that reported above. Ro 46-8443 given after l-NAME did not alter baseline CBF and CD or other hemodynamic variables (Table 2).

Consistent with the data reported above, l-NAME blunted CD responses to adenosine 200 and 500 and slightly reduced (P<0.01) CBF responses to adenosine 500, as reported in Fig. 3. Ro 46-8443 given after l-

<table>
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<th>Control</th>
<th>l-NAME</th>
<th>l-NAME+Ro 46-8443</th>
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<tr>
<td>Left ventricular pressure (mmHg)</td>
<td>111±3</td>
<td>123±4*</td>
<td>128±5</td>
</tr>
<tr>
<td>LV dP/dt (mmHg s&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>2552±96</td>
<td>2444±83</td>
<td>2404±79</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>91±2</td>
<td>101±3*</td>
<td>105±4</td>
</tr>
<tr>
<td>Heart rate (beats min&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>77±2</td>
<td>61±1*</td>
<td>55±2</td>
</tr>
<tr>
<td>Coronary blood flow (ml min&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>40±5</td>
<td>42±5</td>
<td>40±5</td>
</tr>
<tr>
<td>Coronary diameter (mm)</td>
<td>3.56±0.06</td>
<td>3.40±0.07*</td>
<td>3.40±0.07</td>
</tr>
</tbody>
</table>

* P<0.01 vs. previous treatment.

NAME had no further effects on CBF and CD responses to adenosine.

3.4. Blockade of ET<sub>B</sub> receptors

Ro 46-8443 increased LVP (113±3 to 120±4 mmHg, P<0.05) and decreased HR (77±3 to 66±2 beats min<sup>−1</sup>, P<0.01) and CBF (43±5 to 40±4 ml min<sup>−1</sup>, P<0.05). LV dP/dt, MAP and CD were not significantly altered.

Fig. 2. Changes in external coronary diameter (CD, top panels) and coronary blood flow (CBF, bottom panels) caused by ic infusions of adenosine 200 and 500 ng kg<sup>−1</sup> min<sup>−1</sup> before and after ic Ro 61-1790, a selective ET<sub>α</sub> receptor blocker. Ro 61-1790 reduced the increases in CD caused by adenosine 500. * P<0.01 vs. previous treatment (n=8).
Adenosine-induced CD and CBF responses did not statistically differ before and after Ro 46-8443, as reported in Fig. 4.

4. Discussion

The present data highlight the contribution of ET-dependent effects to flow-dependent dilation of large epicardial coronary arteries in conscious dogs. The blunted adenosine-induced flow-dependent dilations of large epicardial coronary arteries after \( \tau \)-NAME were partially restored after ET\(_a\) but not ET\(_\tau\) receptor blockade. Consequently, the use of arginine analogues to suppress NO synthase III activity may not solely reflect the contribution of NO to vasomotor responses but also the influence of ET-dependent effects, magnified by the suppression of NO formation.

Our experimental approach relied on the use of adenosine to elicit sustained dilations of large epicardial coronary arteries. We have previously established that ic doses of adenosine up to 500 ng kg\(^{-1}\) min\(^{-1}\) caused significant flow-dependent dilation of canine coronary epicardial arteries, sensitive to the blockade of NO formation [4]. Preventing the rise in CBF during adenosine administration abolished the increases in CD, consistent with the involvement of elevated shear stress as a pivotal factor leading to endothelial NO formation and vascular relaxation [4]. These observations are in general agreement with earlier studies showing that flow increases were primarily involved in vascular relaxation caused by adenosine in coronary conductance epicardial arteries in dogs [4,26,27] and that NO accounts for vascular relaxation triggered by increases in CBF [2–4].

As expected, blockade of NO formation strikingly impaired the ability of large coronary vessels to dilate as a consequence of flow increases. This conclusion is in line with earlier reports consistently showing that blockade of NO formation blunts flow-dependent dilation to adenosine or after transient coronary arterial occlusions [2–4,18]. The present experiments further establish that suppression of NO formation may not entirely account for the inhibition of flow-dependent responses after an arginine analogue. While our data are consistent with the involvement of NO formation as a primary factor in flow-dependent dilation, the partial recovery of CD dilator responses after blockade of ET\(_a\) receptors indicates that ET-dependent effects were involved and became more apparent after the blockade of
NO formation. Consistent with studies conducted in vitro [6,9–12], our data suggest that an increase in shear stress triggers ET release from large epicardial coronary arteries. NO may normally limit or prevent stimulated ET-dependent effects triggered by increases in shear stress. Removal of this negative feedback by the blockade of NO formation revealed significant ET-dependent effects when shear stress is elevated. In contrast to elevated shear stress conditions, a crosstalk between NO and ET was not apparent under baseline conditions since the blockade of ET\(_A\) receptors caused similar increases in baseline CD when NO formation was normal or impaired. This observation also rules out the possibility that vascular distension secondary to the slight elevation in MAP after \(l\)-NAME magnified or triggered ET activity in those large coronary arteries. The present findings agree with earlier reports showing that a crosstalk between NO and ET is selectively displayed under stimulated conditions [15].

As reported earlier, NO has an inhibitory influence on stimulated ET formation [15,16] as well as a direct antagonistic effect on ET action on smooth muscle cells [17] through a cGMP-dependent process. In fact, stimulated ET production by the porcine aorta is augmented after blockade of NO formation in vitro [15]. The present data are consistent with these earlier studies and with a recent report [20] from our laboratory showing that impairment of acetylcholine-induced dilation of resistance coronary vessels after \(l\)-NAME is partially reversed by the blockade of ET\(_A\) receptors.

In contrast to augmented flow-dependent CD responses with ET\(_A\) receptor blockade after \(l\)-NAME, suppression of ET\(_A\)-dependent influences when NO formation was normal led to slightly reduced CD responses. Consistent with our hypothesis of a crosstalk between NO and ET, blockade of ET\(_A\) receptors prevented the expression of the inhibitory influence of NO on ET activity thereby limiting the flow-dependent increases in CD. These data also imply that the increase in baseline CD caused by Ro 61-1790 cannot account per se for an augmented flow-dependent responses observed after blockade of ET\(_A\) receptors, as we observed when Ro 61-1790 was given after \(l\)-NAME.

In the present study, peripheral hemodynamic effects of \(l\)-NAME and ET receptor antagonists were significant, consistent with recirculation of the blockers. Interestingly enough, pressor effects of \(l\)-NAME were reversed by ET\(_A\) but not not ET\(_\beta\) receptor blockade. This observation agrees with several earlier studies where systemic blockade of NO formation and ET receptors were achieved [28–34]. This apparent crosstalk between NO and ET could not be extended to large epicardial coronary arteries where the
amplitude of CD dilation caused by ET\textsubscript{A} receptor blockade was similar in the face of normal or impaired NO formation [21].

ET\textsubscript{B} receptors have been reported to stimulate NO and prostacyclin (PGI\textsubscript{2}) formation [34–38] from endothelial cells whereas those on smooth muscle cells mediate constriction [23]. To exclude the potentially confounding effect of PGI\textsubscript{2}, animals were pretreated with indomethacin. Under these conditions, blockade of ET\textsubscript{B} receptors failed to influence baseline CD and flow-dependent CD responses with normal or impaired background NO formation. Conceivably, the threshold for ET\textsubscript{B} effects may be higher than for ET\textsubscript{A} responses. Given that ET release in mainly abluminal [39], it is also possible that endogenous ET primarily targets ET\textsubscript{A} receptors located on smooth muscle cells.

In conclusion, blockade of NO formation leads to blunted flow-dependent increases in large epicardial coronary artery diameter partially reversed by ET\textsubscript{A} but not ET\textsubscript{B} receptor blockade. Thus, shear-dependent NO release normally inhibits ET vasoconstriction in large epicardial coronary arteries.

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