Paradoxical coronary microcirculatory constriction during ischemia: a synergic function for nitric oxide and endothelin

Claudia Kusmic, Guido Lazzerini, Flavio Coceani, Renata Barsacchi, Antonio L’Abbate, and Gianmario Sambuceti

Paradoxical coronary microcirculatory constriction during ischemia: a synergic function for nitric oxide and endothelin. Am J Physiol Heart Circ Physiol 291: H1814–H1821, 2006. First published April 28, 2006; doi:10.1152/ajpheart.00220.2006.—A paradoxical microcirculatory constriction has been observed in hearts of patients with ischemia, secondary to coronary stenosis. Here, using the isolated mouse heart (Langendorff), we examined the mechanism of this response, assuming involvement of nitric oxide (NO) and endothelin-1 (ET-1) systems. Perfusion pressure was maintained at 65 mmHg for 70 min (protocol 1), or it was reduced to 30 mmHg over two intervals, between the 20- and 40-min marks (protocol 2) or from the 20-min mark onward (protocol 3). In protocol 1, coronary resistance (CR) remained steady in untreated heart, whereas it progressively increased during treatment with the NO synthesis inhibitor NG-nitro-L-arginine methyl ester (L-NAME) (2.7-fold) or the ET antagonist BQ-610 (2.8 fold). The ETB antagonist BQ-788 had instead no effect (CR progressively increased during treatment with the NO synthesis inhibitor NG-nitro-L-arginine methyl ester (L-NAME) (2.7-fold) or the ET antagonist BQ-610 (2.8 fold). The ETB antagonist BQ-788 had instead no effect during treatment with the NO synthesis inhibitor NG-nitro-L-arginine methyl ester (L-NAME) (2.7-fold) or the ET antagonist BQ-610 (2.8 fold). The ETB antagonist BQ-788 had instead no effect during treatment with the NO synthesis inhibitor NG-nitro-L-arginine methyl ester (L-NAME) (2.7-fold) or the ET antagonist BQ-610 (2.8 fold). The ETB antagonist BQ-788 had instead no effect during treatment with the NO synthesis inhibitor NG-nitro-L-arginine methyl ester (L-NAME) (2.7-fold) or the ET antagonist BQ-610 (2.8 fold). The ETB antagonist BQ-788 had instead no effect.

MATERIALS AND METHODS

Isolated Heart Preparation

Surgical procedures and experimental protocols were approved by the Animal Care Committee of the Ministry of Health and conformed to the “Guiding Principles for Research Involving Animals and Human Beings,” approved by the American Physiological Society. Adult C57BL/6 mice (28 ± 0.7 g body wt) were heparinized (500 U im) 10 min before inducing anesthesia with pentobarbital sodium (40 mg/kg ip). Hearts (weight, 151 ± 4 mg) were excised and placed inside the perfusion line. Volume of effluent was also measured with a calibrated pipette for an additional estimate of coronary flow. Inside the perfusion line, aortic cannula was inserted. The aorta was cannulated with a 20-gauge plastic cannula (40 mg/kg ip). Hearts (weight, 151 ± 4 mg) were excised and placed in ice-cold Krebs-Henseleit bicarbonate solution containing (in mmol/l) 118 NaCl, 24 NaHCO3, 4.7 KCl, 1.2 KH2PO4, 1.2 MgSO4, 2.5 CaCl2, 0.5 EDTA, and 5.5 glucose. The solution had been equilibrated with 95% O2-5% CO2 at pH 7.4. Extraneous tissues were removed, and the aorta was cannulated with a 20-gauge plastic cannula. The heart was then transferred to a modified nonrecirculating Langendorff apparatus where it was allowed to beat spontaneously. Myocardial and buffer temperatures were kept constant at 37°C. Two sidearms in the perfusion line, being located close to the heart inlet, were allowed to switch between two reservoirs set respectively at normal (65 mmHg) and reduced (30 mmHg) pressure. Coronary flow was measured continuously with a flowmeter (model T106, Transonic System, Ithaca, NY) coupled to a 2-N in-line flow probe positioned inside the perfusion line. Volume of effluent was also measured with a calibrated pipette for an additional estimate of coronary flow.

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Coronary resistance was defined as input pressure divided by coronary flow per gram of myocardial tissue (in mmHg·g⁻¹·min⁻¹·ml⁻¹).

**Drugs**

The NO synthesis inhibitors \(N^\text{G}\)-nitro-L-arginine methyl ester (L-NAME) and \(N^\text{G}\)-monomethyl-L-arginine (L-NMMA), the ETA receptor antagonist BQ-610 [homopiperidinyl-carbonyl-Leu-\(d\)-Trp (CHO)-\(d\)-Trp-OH], the ET\(\text{II}\) receptor antagonist BQ-788 (N-cis-2,6-dimethylpyrroloindescarbonyl-L-methylleucyl-\(d\)-1-methoxy carbonyltryptophanyl-\(d\)-norleucine), and Cu/Zn-superoxide dismutase were purchased from Alexis Biochemicals (San Diego, CA). \(N^\text{G}\)-nitro-D-arginine methyl ester (D-NAME), the inactive enantiomer of L-NAME, and catalase were obtained from Sigma (St Louis, MO). Papaverine and mannitol were purchased from Monoic (Venezia, Italy) and Merck (Darmstadt, Germany), respectively. The nonpeptide ETA receptor antagonist 4-(7-ethyl-benzo[1,3]dioxol-5-yl)-1,1-dioxo-2-(2-trifluoromethyl-phenyl)-1,2-dihydro-1H-benzo-[e][1,2]thiazine-3-carboxylic acid potassium (PD-180988) was kindly provided by Pfizer. All drugs were added to the perfusion medium at the time of aorta cannulation and were kept in contact with the preparation for the whole experiment. Concentrations of NO synthase (NOS)- and ET-1 receptor-specific inhibitors were derived from data in the literature (2, 26, 27).

**Protocols**

After an initial 10-min period of stabilization, which was not included in the analysis, the study required continuous monitoring of coronary flow for a 70-min period. Preparations were studied under three conditions: control pressure (protocol 1), transient hypotension (protocol 2), and sustained hypotension (protocol 3). Tables 1–3 report a breakdown of experiments according to the protocol used and the attendant treatment.

**Protocol 1: control pressure.** Preparations were maintained at 65 mmHg input pressure during the entire 70-min experiment and served as a reference.

**Protocol 2: transient hypotension.** Input pressure was switched from 65 to 30 mmHg at the 20-min mark for a period of 20 min, and it was then restored to the initial pressure for the remaining 30 min.

**Protocol 3: sustained hypotension.** Input pressure was switched from 65 to 30 mmHg at the 20-min mark and was kept low for the remaining 50 min.

NO function was evaluated under the three conditions by testing L-NAME (100 μmol/l). Likewise, BQ-610 (0.1 μmol/l) and BQ-788 (1 μmol/l) were used, singly or in combination, to assess ET-1 function. Separate controls for the specificity of drugs included the use of L-NMMA (100 μmol/l), PD-180988 (1 μmol/l), and d-NAME (100 μmol/l); mannitol (20 mmol/l) and the combination of superoxide dismutase (60 U/ml) with catalase (40 U/ml) were tested to neutralize any ROS formed during hypotension. The presence of active vascular tone was ascertained with a bolus injection of papaverine (50 μg) at the 35- and 55-min mark during sustained hypotension (protocol 3) and at the 55-min mark, during the recovery phase, in the transient hypotension experiments (protocol 2). Similarly, papaverine was tested during ETA receptor blockade under normal pressure (protocol 1). To avoid any artifact, all drugs were injected slowly through a catheter connected to a sideline.

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![Fig. 1. Isolated mouse heart. Top: coronary resistance at constant pressure of 65 mmHg in the absence (○) and presence (□) of \(N^\text{G}\)-nitro-L-arginine methyl ester (L-NAME). The specificity of the L-NAME effect was confirmed by the absence of any change with \(N^\text{G}\)-nitro-D-arginine methyl ester (D-NAME; ●) and by its reversal on washout (■, starting at arrow). Bottom: progressive increase in coronary resistance with either BQ-610 (○) or PD-180988 (●) treatment. As expected from an active phenomenon, papaverine (inset, at arrows) reversed the response. BQ-788 had no effect by itself (○), but it reduced the BQ-610-induced vasoconstriction (□).](http://ajpheart.physiology.org/)

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*AJP-Heart Circ Physiol* • VOL 291 • OCTOBER 2006 • www.ajpheart.org
Table 1. Isolated mouse heart preparation at constant pressure: changes in coronary resistance with time and drug treatment (protocol 1)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>0 min</th>
<th>20 min</th>
<th>22 min</th>
<th>40 min</th>
<th>42 min</th>
<th>70 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>5.93±0.26</td>
<td>6.43±0.20</td>
<td>6.46±0.19</td>
<td>6.70±0.14</td>
<td>6.80±0.14</td>
<td>6.90±0.11‡</td>
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<tr>
<td>l-NAME</td>
<td>6</td>
<td>6.47±0.61</td>
<td>12.23±0.84‡</td>
<td>12.93±0.93‡</td>
<td>14.61±1.67‡</td>
<td>15.44±1.48‡</td>
<td>18.92±0.98‡</td>
</tr>
<tr>
<td>D-NAME</td>
<td>6</td>
<td>5.13±0.64</td>
<td>6.72±0.87</td>
<td>6.58±0.81</td>
<td>7.63±0.88</td>
<td>7.74±0.71</td>
<td>8.19±0.48‡</td>
</tr>
<tr>
<td>L-NMMA</td>
<td>2</td>
<td>6.28</td>
<td>11.89</td>
<td>12.30</td>
<td>14.80</td>
<td>18.30</td>
<td></td>
</tr>
<tr>
<td>BQ-610</td>
<td>6</td>
<td>5.66±0.41</td>
<td>11.73±0.63‡</td>
<td>12.04±0.82‡</td>
<td>15.97±2.84‡</td>
<td>16.14±3.25‡</td>
<td>19.80±3.67‡</td>
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<tr>
<td>BQ-180988</td>
<td>6</td>
<td>4.77±0.32</td>
<td>9.29±0.44‡</td>
<td>9.74±0.57‡</td>
<td>14.40±0.43‡</td>
<td>14.71±0.52‡</td>
<td>18.25±0.48‡</td>
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<td>BQ-788</td>
<td>6</td>
<td>4.58±0.21</td>
<td>7.51±0.47</td>
<td>7.85±0.38</td>
<td>9.22±0.41‡</td>
<td>9.11±0.53‡</td>
<td>8.90±0.49‡</td>
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<td>BQ-788 + BQ-610</td>
<td>6</td>
<td>4.31±0.38</td>
<td>9.01±1.36‡</td>
<td>9.20±1.32‡</td>
<td>11.02±1.37‡</td>
<td>10.91±1.47‡</td>
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<tr>
<td>Mannitol</td>
<td>6</td>
<td>6.10±0.32</td>
<td>6.47±0.59</td>
<td>6.61±0.61</td>
<td>7.39±0.95</td>
<td>7.64±0.98</td>
<td>8.26±1.70</td>
</tr>
<tr>
<td>SOD + catalase</td>
<td>6</td>
<td>3.98±0.93</td>
<td>6.00±0.93</td>
<td>6.37±0.88</td>
<td>7.40±1.50</td>
<td>7.39±1.23</td>
<td>5.26±1.80</td>
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</tbody>
</table>

Values are means ± SE (in mmHg·g⁻¹·min⁻¹·ml⁻¹); n, number of hearts. l-NAME, N⁵-nitro-l-arginine methyl ester; D-NAME, N⁵-nitro-D-arginine methyl ester; l-NMMA, N⁵-monomethyl-l-arginine. Perfusion pressure was kept at 65 mmHg and preparations were treated with the drug throughout the whole period of recording. *P < 0.05 vs. 0 time; †P < 0.01 and ‡P < 0.001 vs. untreated hearts; §P < 0.05 vs. BQ-610-treated hearts.

Statistical Analysis

Coronary resistance values were expressed as means ± SE. One-way ANOVA, followed by Bonferroni’s test, for multiple comparisons was used. Differences were considered significant with P < 0.05.

RESULTS

Control Pressure

As shown in Fig. 1 and Table 1, untreated hearts, being perfused at a constant pressure (65 mmHg), showed stable values of coronary resistance, with only a slight upward shift occurring over the entire study period (from 5.93 ± 0.26 to 6.90 ± 0.11 mmHg·g⁻¹·min⁻¹·ml⁻¹) at 0 and 70 min, respectively; P < 0.05). Conversely, when l-NAME was added to the medium, there was a progressive and marked rise in resistance, which attained significance (P < 0.01 vs. untreated heart) already at the 8-min mark (Fig. 1, top). At the peak, coronary resistance increased by about 2.7-fold over control. A comparable increase in resistance was obtained with l-NMMA (≈2.6-fold of control, Table 1). D-NAME, on the other hand, had no significant effect (Fig. 1). Withdrawal of l-NAME from the perfusion fluid interrupted the progression of the vasoconstrictor response, and, in fact, coronary resistance started to reverse toward the control values (Fig. 1).

BQ-610 and PD-180988 enhanced coronary resistance to a similar degree (2.8- and 2.6-fold, respectively; P < 0.001), but the time course of the latter inhibitor was slightly slower (Fig. 1, bottom). This increase was reversed by papaverine, as one would expect from the operation of an active process (Fig. 1, inset). Conversely, BQ-788 had little, if any, effect when tested alone, but it reversed most of the contraction due to BQ-610 (Fig. 1, bottom).

Treatment with either mannitol or superoxide dismutase-catalase did not modify significantly vascular resistance (Fig. 2, top).

Transient Hypotension

Changes in coronary resistance during transient hypotension in the untreated versus treated heart are shown in Fig. 3, top, and Table 2. In the absence of any treatment, hypotension was associated with an increase in coronary resistance. The response was biphasic, with a rapid rise in the first 2 min, followed by a slower progression up to a maximum of 2.2-fold over baseline (Table 2, P < 0.001). Return to normal pressure caused an immediate fall in resistance to a steady, but still elevated (1.7-fold), value (P < 0.01). Partial persistence of the response was due to an underlying vasoconstriction, because papaverine administration at 15 min through the posthypotension period caused a complete reversal (Fig. 3, inset).

The constrictor response to hypotension was completely reversed and, in fact, converted into vasodilation by either ETₐ...
(PD-180988 or BQ-610) or ET\textsubscript{B} (BQ-788) antagonists. As shown in Fig. 3, bottom, all such treatments produced the early increase in coronary resistance noted with protocol 1, but this trend was interrupted by an abrupt fall when reducing the perfusion pressure so that resistance values overlapped with those of control, normotensive preparations. Subsequent return to normal perfusion pressure resulted in a rebound increase in coronary resistance, although up to a value lower than that of the untreated preparation.

L-NAME also converted the response to hypotension from vasoconstriction to vasodilation (Fig. 3, top) and, hence, did not differ in its effect from the ET-1 antagonists. D-NAME, on the other hand, did not affect coronary resistance, whether under normotensive or hypotensive conditions (Fig. 3, top).

The coronary constrictor response to hypotension was also modified by mannitol and the combination of superoxide dismutase with catalase. As shown in Fig. 2, bottom, either treatment blunted the rise in tension, particularly in its early phase, so that the final values achieved (0.7- and 0.6-fold increase for mannitol and superoxide dismutase-catalase, respectively) were lower than control (\(P < 0.01\)). These differences in tension output between treated and untreated preparations persisted upon return to normal pressure.

### Table 2. Isolated mouse heart preparation: change in coronary resistance during transient hypotension and its modification by drug treatment (protocol 2)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>0 min</th>
<th>20 min</th>
<th>22 min</th>
<th>40 min</th>
<th>42 min</th>
<th>70 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>6.59±0.25</td>
<td>7.59±0.27</td>
<td>10.37±0.56†</td>
<td>14.50±0.83†</td>
<td>11.12±0.65*</td>
<td>11.48±0.60*</td>
</tr>
<tr>
<td>L-NAME</td>
<td>8</td>
<td>6.04±0.27</td>
<td>13.71±1.18§</td>
<td>10.00±0.90</td>
<td>8.87±0.47§</td>
<td>14.86±2.12‡</td>
<td>21.67±2.12‡</td>
</tr>
<tr>
<td>D-NAME</td>
<td>6</td>
<td>6.62±0.30</td>
<td>7.76±0.55</td>
<td>10.51±0.63</td>
<td>15.98±2.93</td>
<td>11.47±1.58</td>
<td>13.75±1.97</td>
</tr>
<tr>
<td>BQ-610</td>
<td>6</td>
<td>5.96±0.89</td>
<td>13.26±1.43§</td>
<td>9.63±1.12</td>
<td>11.04±1.14‡</td>
<td>17.30±3.00†</td>
<td>14.89±1.53‡</td>
</tr>
<tr>
<td>BQ-180988</td>
<td>6</td>
<td>4.98±0.29</td>
<td>8.95±0.40</td>
<td>6.23±0.45§</td>
<td>7.90±0.45§</td>
<td>11.31±0.57</td>
<td>11.87±0.65</td>
</tr>
<tr>
<td>BQ-788</td>
<td>6</td>
<td>5.11±0.35</td>
<td>9.39±0.54‡</td>
<td>5.32±0.33§</td>
<td>6.18±0.39§</td>
<td>11.31±1.28</td>
<td>13.21±0.99</td>
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<tr>
<td>Mannitol</td>
<td>6</td>
<td>5.03±0.53</td>
<td>7.02±0.94</td>
<td>7.41±0.95§</td>
<td>11.36±0.63‡</td>
<td>9.60±0.67‡</td>
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<tr>
<td>SOD + catalase</td>
<td>6</td>
<td>4.37±0.14</td>
<td>6.34±0.70</td>
<td>6.84±0.84§</td>
<td>9.66±1.38‡</td>
<td>7.43±0.43§</td>
<td>6.78±0.58‡</td>
</tr>
</tbody>
</table>

Values are means ± SE (in mmHg·g·min·ml\(^{-1}\)); n, number of hearts. Perfusion pressure was reduced from 65 to 30 mmHg between the 20- and 40-min marks. Drug treatment continued throughout recording, regardless of level of perfusion pressure. *\(P < 0.05\) and †\(P < 0.001\) vs. 0 time; ‡\(P < 0.01\) and §\(P < 0.001\) vs. untreated hearts.

Fig. 3. Isolated mouse heart. Increase in coronary resistance upon reducing transiently the perfusion pressure from 65 to 30 mmHg (○). Top: vasoconstriction is converted into vasodilation upon treatment with L-NAME (●), whereas the response remains unchanged in the presence of D-NAME (■). Bottom: conversion of hypotension-induced vasoconstriction into vasodilation with BQ-610 (▲), PD-180988 (♦), and BQ-788 (○). Note the vasorelaxant effect of papaverine on posthypotension baseline (inset).
**Sustained Hypotension**

As expected from the experiments with transient hypotension, in the untreated heart a reduction in perfusion pressure over an extended period resulted in a rise in coronary resistance (Fig. 4 and Table 3). The response became progressively greater with time until, at the end of the recording period, values exceeded controls by 2.3-fold \((P < 0.01)\). The vasoconstrictive nature of this phenomenon was confirmed with papaverine, which, as evident from Fig. 4, inset, abated the tone to baseline.

Like transient hypotension, a prolonged hypotension resulted in a fall, rather than an increase, of coronary resistance in preparations pretreated with either 1-NAME or BQ-610 (Fig. 4). L-NMMA behaved as 1-NAME in this respect (Table 3).

**DISCUSSION**

Our aim was to ascertain whether the “paradoxical” coronary vasoconstriction observed during myocardial ischemia in patients with severe coronary stenosis is the consequence of an endothelial dysfunction or rather a normal response to a reduction in distal pressure. Herein, we provide three main findings: 1) a sudden decrease in perfusion pressure is consistently associated with a vasoconstriction in the isolated, nonworking beating heart from healthy, wild-type animals; 2) the vasoconstrictive response to hypotension requires the integrity of both NO and ET-1 systems and the likely contribution of ROS being generated by the reduction of flow; and 3) the level of coronary pressure conditions changes, resulting from the interference with the NO and ET-1 systems, with either treatment causing vasoconstriction and vasodilation, respectively, at normal (65 mmHg) and reduced (35 mmHg) values.

**Coronary Pressure and Vasomotor Tone**

In the untreated heart, a sudden reduction in the perfusion pressure caused a biphasic rise in coronary resistance, characterized by a rapid slope in the first 2 min, followed by a slower progression. Such an increase subsided when normal pressure was restored after 20 min, although baseline values were not achieved. In our ex vivo preparation (beating heart with empty cavities), increased vascular resistance during hypotension does not result, as it may happen in vivo (10), from a rise in extravascular compressive forces secondary to increased left ventricular preload and impaired relaxation during ischemia. More importantly, in our model, the active character of the elevated resistance was confirmed by its reversal upon either the interference with locally formed vasoactive agents or treatment with papaverine.

Vasoconstriction to reduced pressure may seem paradoxical insofar as it challenges the conventional concept of coronary autoregulation. However, microvascular tone regulation has not been studied exhaustively in instances in which arterial pressure falls below the autoregulatory range (8). Nevertheless,
the concept of autoregulation has been extended to predict a maximal dilation of coronary microvessels in the myocardium being rendered ischemic by a severe stenosis. This assumption is still largely accepted, despite a number of experimental (6, 12, 15, 16, 21) and clinical (24, 30, 32, 34) studies documenting submaximal vasodilation or a full-fledged vasoconstriction in response to increased myocardial oxygen demand while blood flow is limited by coronary obstruction. Our study proves that a vasoconstrictive response to hypotension occurs with the heart from healthy animals, hence excluding atherosclerotic impairment of vascular function as a prerequisite.

**ET and NO Vis-à-Vis Arterial Pressure and Vasomotor Tone**

Endothelium-related vasoactive substances seemingly play a special and unexpected role in the vasomotor response to reduced pressure. Interference with ET-1 receptor subtypes or NOS function restored the autoregulatory response to pressure changes being predicted by the conventional scheme, i.e., vasodilation and vasoconstriction, respectively, with reduction and increase in pressure. Accordingly, the treated hearts reached the lowest resistance during hypotension, whereas the reverse occurred with the untreated ones. Specificity of changes with NOS inhibition is supported by the coincidence of actions between l-NNAME and l-NMMA, the lack of effect of d-NNAME, and the reversal of responses upon discontinuation of treatment. On the other hand, manipulations of the ET-1 system find their validation in the congruence of results with structurally different ETA antagonists (i.e., BQ-610 and PD-180988) as well as in the dependence of such responses on the viability of ETB receptors. Indeed, the response of coronary resistance to pressure changes was remarkably similar with ETa and NOS inhibition. This coincidence of effects implies that NO- and ET-1/ETa-linked events are concerted steps in the process underlying the vasomotor response to pressure changes. Moreover, the same data show that the sign of NO- and ETa-mediated actions is critically dependent on the prevailing hemodynamic condition.

This synergism between NO and ET-1 in the coronary response to pressure changes outwardly stands against the large literature proving opposing functions for the two agents (28). Nevertheless, while studying the immediate response to ET-1 in porcine coronary microcirculation, Baydoun and coworkers (3) already reported an early transient vasodilation dependent on NO availability.

The present data do not provide a conclusive explanation for the synergy between NO and ETa-based ET-1 actions in sustaining the constrictor response of the coronary microvasculature to hypotension. However, it should be noted that this response was blunted by ROS scavengers, which by themselves were without effect in the normotensive state. This observation, being in line with the notion that ischemia promotes ROS formation, points to the possibility of NO being converted by ROS to a reactive species with constrictor properties (peroxynitrite?). Added strength to this hypothesis comes from the knowledge that NOS, specifically its inducible form, is also upregulated by ischemia (19). Hence, a reactive nitrogen oxide species could initiate a sequence of events including, in the following order, increased metalloproteinase-2 activity, directly or through the inhibition of tissue inhibitor of metalloproteinases-2 (5, 11, 13, 35), and activation of the ET-1 system with the greater release of the peptide (9), accelerated cleavage of the peptide to a more powerful derivative (i.e., ET-1, 1 to 32) (11), or perhaps some upregulation of ET-1 receptors (29). Against this scenario, inhibition of NOS would interrupt the sequence at its start, whereas BQ-610 would suppress the ultimate effector.

Another apparent paradox in our study is the progressive increase of coronary resistance in the normotensive preparation upon treatment with ETa antagonists. A similar finding, however, has been reported by others and has been ascribed to the removal of an ETa-based negative control on ET-1 acting on a subset of ETb receptors with constrictor function (i.e., ETb2) (36). Our data are consistent with the latter explanation because the combination of ETb with ETa antagonists greatly curtailed the constriction to the ETa antagonist alone.

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**Table 3. Isolated mouse heart preparation: change in coronary resistance during sustained hypotension and its modification by drug treatment (protocol 3)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>0 min</th>
<th>20 min</th>
<th>22 min</th>
<th>40 min</th>
<th>42 min</th>
<th>70 min</th>
</tr>
</thead>
<tbody>
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<td>Control</td>
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<td>5.15±0.50</td>
<td>7.11±0.76</td>
<td>9.65±1.46*</td>
<td>13.32±1.96†</td>
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<td>l-NNAME</td>
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<td>9.08±0.68‡</td>
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<td>BQ610</td>
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<td>9.86±1.52‡</td>
<td>10.01±1.52‡</td>
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</tbody>
</table>

Values are ± SE (in mmHg·g·min⁻¹·ml⁻¹); n, number of hearts. Perfusion pressure was reduced from 65 to 30 mmHg at the 20-min mark. Drug treatment continued throughout the recording, regardless of level of perfusion pressure. *P < 0.05 and †P < 0.001 vs. 0 time; ‡P < 0.05 and §P < 0.001 vs. untreated hearts.
ing this line of reasoning, one may also surmise that the negative feedback of ETA on ETB2-mediated action subsides during hypotension so that the full constrictor potential of ET-1 may unfold. On the other hand, the powerful vasoconstrictor effect of l-NAME in normotensive preparations can be safely ascribed to the removal of tonically acting NO (14).

In brief, we propose that under normal pressure, the coronary vasculature is kept relaxed by the combined impact of NO and ETA activation, with the latter exerting a negative control on ETB2 rather than a direct effect on muscle. During hypotension, vasoconstrictor influences prevail through the ROS-induced conversion of NO to some reactive species and the dual activation of ETA and ETB2 receptors by ET-1 (Fig. 5).

Limitations

The idea for the study originated from the observation of a “paradoxical” coronary vascular constriction in patients with ischemia secondary to severe coronary stenosis. The study itself was designed to reproduce this finding with the specific aim of testing the pressure/tone relationship in the control of coronary blood flow. However, differences between the ex vivo mouse model and the patient condition pose a limit on the direct extension of our results to the clinical setting. The heart was perfused with an artificial medium and not with blood. Accordingly, our preparation necessitated a high flow rate with the attendant possibility of basal relaxation preventing any further dilation with reduced pressure. However, the preparation at start, under normal conditions of pressure, does not show maximal vasodilation, because an adenosine A2 receptor agonist may decrease coronary resistance by over 30% (data not shown). On the other hand, suppression of the vasoconstrictive response to hypotension by diverse pharmacological manipulations, including the use of papaverine, documents the existence of a contractile drive being independent from baseline tone. A higher basal tone would have possibly resulted in total interruption of flow during hypotension.

Extramural determinants of coronary vascular resistance were not specifically considered, but, lacking an intraventricular pressure within the beating heart, they may be linked only to heart rate and the contractile state of the myocardium. During hypotension, however, heart rate abates, and, hence, any change in compressive force would go into a direction opposite to that expected from a factor driving vascular resistance upward. This conclusion is strengthened further by the observation that papaverine reduces vascular resistance without any change in heart rate and, by inference, in contractility. Moreover, the absence of blood in our system excluded any microvascular obstruction by white blood cells, that is, a possible complication of ischemia causing in vivo an increase of coronary resistance independently from vasomotor tone (18, 22). Likewise, heart denervation ruled out any contribution of neural reflexes in the vascular reactivity to pressure changes. Nevertheless, despite the limitations, findings in our model coincide with the clinical situation and lead us to conclude that vasoconstriction in the low-pressure range represents a basic mechanism of microvascular control with a pivotal role in the pathophysiology of ischemia. Still outstanding remain the nature and intramyocardial arrangement of the vascular microdomains involved in this adaptive change (see Ref. 17). In conclusion, our results agree with previous observations concerning a marked microvascular constriction during cardiac ischemia. The occurrence of a constriction to coronary hypotension in the isolated heart of healthy mice strongly supports the concept that this response represents a natural process of adaptation. When we consider the large evidence of heterogeneity of flow (33), flow reserve (1), and metabolism (23) during ischemia and also the dependence of the perfused tissue mass on the coronary pressure distal to a severe stenosis (31), it is conceivable that this response reflects a mechanism apt to preserve capillary pressure in certain parts of the vascular bed while excluding others from perfusion. The endothelium appears to play a pivotal role in the vascular response to coronary hypotension through the generation of ROS that promote a cooperative rather than an antagonistic interaction between the NO and ET-1 systems. A mechanism is, therefore, postulated whereby intravascular pressure exerts a direct control on endothelial function opposite in sign to the myogenic reflex.

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