Reduced NO-dependent arteriolar dilation during the development of cardiomyopathy

DONG SUN, AN HUANG, GONG ZHAO, ROBERT BERNSTEIN, PAUL FORFIA, XIAOBIN XU, AKOS KOLLER, GABOR KALEY, AND THOMAS H. HINTZE

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Sun, Dong, An Huang, Gong Zhao, Robert Bernstein, Paul Forfia, Xiaobin Xu, Akos Koller, Gabor Kaley, and Thomas H. Hintze. Reduced NO-dependent arteriolar dilation during the development of cardiomyopathy. Am. J. Physiol. Heart Circ. Physiol. 278: H461–H468, 2000.—Our previous studies have suggested that there is reduced nitric oxide (NO) production in canine coronary blood vessels after the development of pacing-induced heart failure. The goal of these studies was to determine whether flow-induced NO-mediated dilation is altered in coronary arterioles during the development of heart failure. Subependymal coronary arterioles (basal diameter 80 µm) were isolated from normal canine hearts, from hearts with dysfunction but no heart failure, and from hearts with severe cardiac decompensation. Arterioles were perfused and increasing flow or administered agonists with NO flow in vitro. In arterioles from normal hearts, flow increased arteriolar diameter, with one-half of the response being NO dependent and one-half prostaglandin dependent. Shear stress-induced dilation was eliminated by removing the endothelium. Arterioles from normal hearts and hearts with dysfunction but no heart failure responded to increasing shear stress with dilation that reached a maximum at a shear stress of 20 dyn/cm². In contrast, arterioles from failing hearts showed a reduced dilation, reaching only 55% of the dilation seen in vessels of normal hearts at a shear stress of 100 dyn/cm². This remaining dilation was eliminated by indomethacin, suggesting that the NO-dependent component was absent in coronary microvessels after the development of heart failure. Similarly, agonist-induced NO-dependent coronary arteriolar dilation was markedly attenuated after the development of heart failure. After the development of severe dilated cardiomyopathy and heart failure, the NO-dependent component of both shear stress- and agonist-induced arteriolar dilation is reduced or entirely absent.

prostaglandins; compensated and decompensated heart failure; shear stress; bradykinin

THE ROLE OF ALTERATIONS IN nitric oxide (NO) production in myocytes (1, 3, 7) or coronary blood vessels (5, 14, 26, 28) during the development of heart failure is still controversial. Although a number of studies suggest that myocyte (1, 3, 7) or blood vessel (8, 19) NO production may contribute significantly to the reduced inotropic effects associated with heart failure, studies using a well-controlled model of dilated cardiomyopa-thy have suggested that production of NO is reduced after the development of heart failure. For instance, we have shown that NO-dependent large coronary artery dilation is reduced after the development of pacing-induced heart failure (28), as is the NO-dependent coronary vasodilation caused by activation of the Bezold-Jarisch reflex (33). Furthermore, there is a significant reduction in both the mRNA and protein for endothelial constitutive NO synthase (eNOS) after heart failure in freshly harvested aorta endothelium when normalized to the expression of either glyceraldehyde-3-phosphate dehydrogenase or von Willebrand's factor (24). In addition, Recchia et al. (20) from our laboratories have shown that nitrate production across the heart is significantly reduced only after cardiac decompensation.

Recently, we also have shown in sieved coronary microvessels from the failing canine (33) and human (9) heart that nitrite production stimulated by ACh and bradykinin is significantly reduced after heart failure. However, these studies used a preparation of blood vessels of heterogeneous types, including small arterioles, attached capillaries, and venules. Additionally, although that preparation selects for microvessels <100 µm in diameter over myocytes, routinely there are fragments of cardiac myocytes and substantial amounts of connective tissue present that make conclusions based on calculations of NO production normalized to wet weight somewhat tenuous. Whereas studying sieved coronary blood vessels may be an expedient way of determining agonist-induced NO production, using them to assess changes in flow-mediated dilation is not possible. This is of critical importance because the physiological stimulus for NO production may be moment-to-moment changes in coronary blood flow velocity and/or shear stress (11).

To directly address the alterations in NO production in coronary microvessels during the development of cardiomyopathy in the dog, we applied techniques previously used by us (12, 13, 25) to perfuse single arterioles. The goals of our study were to determine the time course for alterations in NO-dependent responses, particularly flow-mediated coronary microvessel dilation, during the development of cardiac decompensation and to determine the relative contribution of NO and prostaglandins to dilation in coronary microvessels from the normal, compensated, and failing canine heart.
MATERIALS AND METHODS

Surgical preparation. Twenty-six mongrel dogs (weight 20–35 kg) were chronically instrumented for measurement of systemic hemodynamics (20, 23, 28). Briefly, dogs were premedicated with acepromazine (0.3 mg/kg im) and anesthetized with pentobarbital sodium (25 mg/kg iv). A thoracotomy was performed in the left fifth intercostal space using sterile surgical technique. Tygon catheters (Cardiovascular Instruments) were placed in the descending thoracic aorta and left atrial appendage for measurement of pressures. A solid-state pressure gauge (P 6.5 Königsberg Instruments) was placed in the apex of the left ventricle for the measurement of left ventricular (LV) systolic pressure (LVSP), and LV end-diastolic pressure (LVEDP). A pair of pacing electrodes was sutured on the left ventricle for controlling heart rate. The chest was closed in layers. The wires and the catheters were run subcutaneously and exited from the back of the dog's neck. The dog carried an external pacemaker (model EV4543, Pace Medical, Waltham, MA) in a vest.

Development of heart failure. After 10–14 days of recovery, hemodynamic baseline was recorded and the heart was paced at 210 beats/min for 3 wk. The pacing rate was then increased to 240 beats/min for an additional week to cause heart failure (n = 9). Ten dogs that were instrumented for 5 wk but not paced were used as a normal control group; seven dogs whose hearts were paced for 3 wk were used to determine microvessel responses after compensated cardiac dysfunction. We have used these techniques previously (2, 20, 24, 28, 29, 33).

All studies were approved by the Institutional Animal Care and Use Committee of New York Medical College and conform to the current National Institutes of Health guidelines and the guidelines of the American Physiological Society for the care and use of laboratory animals.

Vessel preparation. After all hemodynamic measurements were completed, the dogs were anesthetized. The heart was then quickly removed from the chest and a piece of left ventricular muscle, then quickly removed from the chest and a piece of left ventricular muscle, then quickly removed from the chest and a piece of left ventricular tissue was cut from the LV free wall (2–3 cm²), was cut from the LV free wall and placed in cold (–4°C) MOPS-buffered (pH 7.4) physiological salt solution (PSS) in a dissecting dish. PSS contained (in mM) 145 NaCl, 5 KCl, 2 CaCl₂, 1 MgSO₄, 1 NaH₂PO₄, 5 dextrose, 2 pyruvate, 0.02 EDTA, and 3 MOPS. The muscle was then pinned to the bottom of the silicone-lined base of the dissecting dish.

With the use of microscissors and an operating microscope (Olympus, Lake Success, NY), segments of subepicardial arteries, ~1 mm in length, were separated from the adhering cardiac muscle by careful cutting and transferred to a vessel chamber containing Krebs bicarbonate-buffered PSS at room temperature. The vessel chamber contained two glass microcannulas, which were connected with silicone tubing to two pressure-servo syringe systems (Living Systems, Burlington, VT). The system was arranged with mirror symmetry so that the axis of symmetry was located perpendicular to the middle of the arteriolar segment. This resulted in equal resistance of the two sides of the system. The vessel chamber was connected to a reservoir through a suction pump. The total volume of PSS in the system was 100 ml suffused at a rate of 40 ml/min during the experiment.

The PSS used to perfuse the arteriole contained (in mM) 118 NaCl, 5 KCl, 2.5 CaCl₂, 1 MgSO₄, 1 KH₂PO₄, 10 glucose, 24 NaHCO₃, and 0.02 EDTA and was equilibrated with 21% O₂–5% CO₂ balance N₂, at pH 7.4. After the proximal end of the arteriole was mounted to the inflow cannula and secured with a suture, the pressure was raised to 20 mmHg to flush and clear the vessel of clotted blood. The distal end of the arteriole was then mounted to the outflow cannula. After several minutes of perfusion, the distal outflow cannula was closed and pressure was slowly increased to 60 mmHg. The vessel was then warmed slowly to 37°C and allowed to equilibrate for 60 min (YSI Temperature Controller, Yellow Springs Instrument, Yellow Springs, OH). The diameter of vessels in various experimental conditions was measured with an image-shearing device (IPM model 908, San Diego, CA) and recorded with a chart recorder (Graphtec Multicorder MC6625, Tokyo, Japan).

Experimental protocols. Isolated coronary arterioles of dogs were equilibrated at 60 mmHg in a no-flow condition for 1 h to allow the vessel to develop spontaneous tone. At the conclusion of the experiments, passive diameter of the arterioles, at 60 mmHg, was obtained in calcium-free PSS containing EGTA (1 mM).

Flow-induced dilation. After the equilibration period, flow-diameter relationships were obtained under control conditions in arterioles from hearts of normal dogs and from hearts paced for 3 or 4 wk. Perfusion flow was increased from 0 to 25 µl/min in steps of 5 µl/min. Flow was established at a constant intravascular pressure (60 mmHg) by changing proximal and distal pressures to an equal degree but in opposite directions. Flow was measured by a ball flowmeter (FL-300, OMEGA Engineering, Stamford, CT). Each flow step was maintained for 3–5 min to allow the vessels to reach steady-state conditions before the diameter of arterioles was measured.

The role of nitric oxide in flow-induced dilation was assessed by using Nω-nitro-L-arginine (L-NNA; 10⁻⁴ M), an inhibitor of NO synthesis. After control flow-diameter curves were obtained, the vessels were incubated with L-NNA. After a 30-min period, changes in diameter in response to step increases in perfusate flow were then reassessed.

The role of prostaglandins in flow-induced arteriolar dilation was assessed by inhibition of cyclooxygenase. After control responses were obtained, indomethacin (Indo; 10⁻³ M) was added to the perfusion solution. Thirty minutes later, flow-diameter relationships were once more determined. After responses were obtained in the presence of either L-NNA or Indo, the combined effect of the two inhibitors on the flow-diameter relationship was determined. In approximately one-half of the experiments, first L-NNA and then Indo was administered; in the remaining experiments, the inhibitors were administered in reverse order. In separate experiments, flow-induced dilation was also assessed before and after endothelium removal. Removal of endothelium was accomplished by perfusion of air into the vessel lumen as described previously (12, 13).

Vasodilator responses. The function of arteriolar endothelium and smooth muscle was assessed with the use of various concentrations of the endothelium-dependent agents ACh (10⁻⁹–5 × 10⁻⁸ M) and bradykinin (10⁻¹⁰–10⁻⁹ M), and the endothelium-independent agent adenosine (10⁻⁵–5 × 10⁻⁵ M). Responses to vasoactive agents were assessed at 60 mmHg of perfusion pressure and in no-flow conditions. All drugs were added to the vessel chamber (outside the blood vessel), and final concentrations are reported. After the peak response to each drug was reached, the vessel chamber was flushed with PSS.

Statistical analysis and calculations. Data are reported as means ± SE. Differences from control were analyzed using ANOVA for repeated measures and a Dunnett’s test. P < 0.05 from control was considered significant. Shear stress was calculated using the formula 4πQ/r³, where r is the radius of the perfusate (0.007 mm at 37°C), Q is the perfusate flow, and r is the vessel radius. WSS⁺⁺⁺, the wall shear stress required to dilate arterioles to 50% of the maximum diameter.
that can be obtained by increases in perfusate flow, was also calculated.

RESULTS

The designation “normal” in the text and Figs. 1–3 denotes blood vessels from hearts without pacing, whereas “control” denotes the same blood vessel before drug treatment or removal of the endothelium.

Effects of chronic LV pacing on hemodynamics. Table 1 summarizes the alterations of hemodynamics after LV rapid pacing. In 10 dogs, a 5-wk period of instrumentation without pacing had no significant effect on hemodynamics. After 4 wk of pacing, all dogs developed severe congestive heart failure, accompanied by edema, dyspnea, and ascites. LVSP, the first derivative of LV pressure with respect to time. *

Table 1. Effects of left ventricular pacing on hemodynamics of conscious dogs

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>3 wk</th>
<th>4 wk</th>
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<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>134 ± 2</td>
<td>113 ± 3*</td>
<td>106 ± 4*</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>7.4 ± 1.0</td>
<td>18 ± 2*</td>
<td>27 ± 2*</td>
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<tr>
<td>LV dP/dt, mmHg/s</td>
<td>2.946 ± 0.110</td>
<td>1.624 ± 78*</td>
<td>1.383 ± 86*</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>101 ± 2</td>
<td>90 ± 3*</td>
<td>86 ± 2*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>77 ± 6</td>
<td>114 ± 7*</td>
<td>126 ± 5*</td>
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Values are means ± SE. Control values were recorded within 10–14 days after dogs were instrumented. Values for pacing for each group are the last measurements recorded at the 3rd or 4th week of pacing. LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; LV dP/dt, first derivative of left ventricular pressure with respect to time. *P < 0.05 compared with corresponding control value.

Mechanisms of flow-induced dilation. Endothelium removal abolished the dilation to increases in flow. In the presence of l-NNA or Indo, the flow-diameter curves were still significantly different from control (Fig. 2A). At a flow of 25 µl/min, l-NNA or Indo decreased flow-induced dilation by 34% or 68% (P < 0.05), respectively, whereas combined application of both l-NNA and Indo resulted in an additional, significant (P < 0.05) reduction of flow-induced dilation (86% reduction from control).

Effects of LV pacing on flow-induced dilation. The arteriolar diameter, as a function of perfusate flow, was obtained in coronary arterioles from normal dogs, those with dysfunction but no heart failure, and those with overt congestive heart failure (Fig. 1A). There was a significant difference between the diameters of arterioles from normal hearts and those from failing hearts at corresponding flow values, indicating that coronary arterioles from dogs with pacing-induced heart failure have an impaired ability to dilate. The maximum dilation in arterioles from failing hearts was 45% less than that in arterioles from normal hearts. After 3 wk of pacing, coronary arterioles dilated to increases in flow and the maximum dilation was not significantly different from that obtained in coronary arterioles from normal hearts (P > 0.05) but was significantly different in arterioles from dogs with heart failure (P < 0.05).

To compare flow-induced dilation in all three groups, calculated wall shear stress versus change in diameter is shown in Fig. 1B. In arterioles from normal hearts or hearts with 3 wk of pacing, the maximum dilation of arterioles induced by a perfusate flow of 25 µl/min was not significantly different. This resulted in a low, maintained wall shear stress in coronary arterioles from normal dogs and those from dogs with 3 wk of pacing, whereas in arterioles from dogs with heart failure, the reduced flow-induced dilation yielded a higher level of shear stress, ≤100 dyn/cm² at a flow of 25 µl/min. The value of wall shear stress at the half-maximal change in diameter (WSS50) in arterioles from dogs with heart failure was significantly greater than that in arterioles from normal dogs or from dogs with 3 wk of pacing (77 vs. 18 or 18 dyn/cm², respectively). The WSS50 in vessels from normal hearts and those from dogs with 3 wk of pacing were not significantly different.

Mechanisms of impaired flow-induced dilation after heart failure. l-NNA did not significantly reduce flow-induced dilation after the development of heart failure (P > 0.40), whereas Indo inhibited the dilation almost to a similar extent as in normal dogs.
completely (P < 0.05, Fig. 2B). L-NNA and Indo together elicited a further significant suppression of the dilation. Removal of the endothelium essentially abolished flow-dependent dilation. Further calculation and analysis indicated that the flow-diameter curve in vessels of dogs with heart failure was not significantly different from the dilation after L-NNA in arterioles from normal hearts.

Effects of LV pacing on vasodilator responses. Changes in diameter of the arterioles of all three groups in response to various agonists were determined in the presence of a constant intravascular pressure (60 mmHg) and in a no-flow condition. To compare differently sized vessels, the normalized responses are reported (peak changes in diameter of arterioles are divided by the passive diameter at 60 mmHg of intravascular pressure).

There were no significant differences in arteriolar dilation to ACh (Fig. 3A) between arterioles from normal hearts and those with 3 wk of pacing, whereas after heart failure, dilations of arterioles to 10^{-9} M ACh were significantly decreased (46%, P < 0.05). Compared with dilations in vessels of normal dogs, dilations to bradykinin (Fig. 3B) in arterioles from hearts paced for 3 wk were significantly decreased only at a concentration of 10^{-9} M. In contrast, dilations to bradykinin in arterioles from failing hearts were significantly decreased at all three concentrations tested. Also, L-NNA (10^{-4} M) reduced the coronary arteriolar dilation to bradykinin (10^{-9} M) by 62 ± 13% in normal dogs and by 39 ± 14% in dogs with heart failure when applied to the outer surface of the blood vessels in vitro.

Dilations to various doses of adenosine were not significantly different among the vessels of the three groups of dogs (Fig. 3C).

**DISCUSSION**

In the current study we have applied techniques used by us previously in rats (11–13, 25) to perfuse isolated canine coronary microvessels to assess the role of vascular NO production in the progressive development of severe decompensated heart failure. The most significant findings of our study were that 1) microvessels from the coronary circulation of normal dogs respond to agonists and increasing flow with substantial dilation in vitro; 2) the dilations to increased flow are both NO and prostaglandin dependent; 3) these dilations are not substantially altered after 3 wk of pacing when there is dysfunction but no clinical evidence of heart failure; and 4) after the development of severe dilated cardiomyopathy and heart failure, there is a selective diminution in NO-dependent vasodilation to both flow and agonists. Whereas our previous studies in vivo using piezoelectric crystals to measure large coronary artery diameter (28) indicated a reduction in NO-dependent vasodilation to both flow and agonists, there is a selective diminution in NO-dependent vasodilation to both flow and agonists. The present work indicates the first attempt to directly study a well-defined microcirculatory element after the controlled induction of cardiac failure and the first reported study of canine coronary microvessel reactivity in vitro.
In initial studies we partially characterized the properties of small canine coronary arterioles. These studies indicated that canine coronary arterioles responded to agonists used to cause vasodilation, including ACh, bradykinin, and adenosine. A portion of the dilation to agonists, with the exception of dilation to adenosine, was eliminated after incubation with L-NNA or removal of the endothelium using air (25).

Coronary arterioles from the normal dog heart were sensitive to increases in shear stress, reaching maximum dilation (90% of passive diameter) at a shear stress of 20 dyn/cm², a near doubling of the basal arteriolar diameter. A portion of the flow-mediated dilation was inhibited after L-NNA was administered, and the remaining portion was eliminated after Indo was administered. These results indicate that both NO and vasodilator prostaglandins are involved in canine coronary arteriolar dilation during increases in flow. Furthermore, the combination of L-NNA and Indo or removal of the endothelium essentially eliminated the vasodilation to increased shear stress, indicating that additional vasodilator mechanisms play, at best, a minor role. The results are in contrast to a previous report by Kuo et al. (15), who demonstrated in microvessels from the porcine coronary circulation that flow-mediated dilation is mediated solely by the release of NO.

The primary goal of this study, however, was to determine the time course and magnitude of the alterations in coronary microvessel control that occurs during the development of dilated cardiomyopathy. To this end, we used a unique model of pacing-induced heart failure that had been previously and extensively characterized in our laboratory (2, 5, 6, 17, 20, 24, 28, 33). In this model, heart failure is produced by LV pacing at 210 beats/min for 3 wk and then increased in a stepwise fashion to 240 beats/min until clinical signs of heart failure appear and LVEDP is >25 mmHg. Using these methods previously, we found a consistent time course leading to the development of severe decompensated heart failure that occurs in 31 ± 3 days and is distinctly different from that in models using a constant and high heart rate in which heart failure appears anywhere from 8 to 40 days of pacing. In the current study we found that after 3 wk of pacing and with the pacemaker turned off, EDP was elevated and accompanied by tachycardia and a reduction in LV dP/dt but no clinical signs of heart failure. At this time, the vasodilation to both NO-dependent and -independent agents was essentially unchanged. It is also of note that increases in flow still led to maximum dilation at a shear stress of ~20 dyn/cm². Thus even in a state of compensated cardiac dysfunction, NO-mediated control of coronary vascular regulation is unchanged. It is important to note that the maximum dilation to bradykinin (10⁻⁹ M) in microvessels was reduced after 3 wk of LV pacing, and this contrasts with the lack of alterations to either shear stress or ACh. This may indicate an altered coupling of the bradykinin receptor to NOS or possibly a statistical anomaly.

Recent data suggested that the signal between changes in flow or agonists and NO production may be different (18), with the former being Ca²⁺ independent but the latter Ca²⁺ dependent. Be that as it may, our data suggest that neither of these coupling mechanisms are consistently affected by 3 wk of LV pacing, and this contrasts with the lack of alterations to either shear stress or ACh. This may indicate an altered coupling of the bradykinin receptor to NOS or possibly a statistical anomaly.

The response to adenosine was not altered after 3 wk of pacing, suggesting that the ability of vascular smooth muscle to relax via cAMP-mediated mechanisms was not altered.

In marked contrast to the response of coronary microvessels to flow and agonists after 3 wk of LV pacing, there were dramatic and selective alterations in NO-mediated dilations after the development of heart failure, in that there were substantial, 30–40% reductions in dilations to ACh and bradykinin. The effect of flow- and/or shear stress-induced dilations of coronary arterioles during the development of pacing-
induced heart failure may have been even greater. There was only a minimum dilation at shear stresses ≤20 dyn/cm² and only 50% of maximal dilation at a shear stress of 100 dyn/cm². Thus a fivefold greater shear stress is required to dilate arterioles from dogs in heart failure than arterioles from normal canine hearts or hearts with 3 wk of pacing. To buttress these findings, there was no difference in the responses of vessels to adenosine after the development of heart failure. Thus the segment of the coronary circulation that we studied was still capable of dilation by two mechanisms, via cAMP or cGMP. The finding that both agonist (ACh and bradykinin)-induced and flow- and/or shear stress-induced dilation were reduced after the development of heart failure indicates that it is most likely not a transduction mechanism that is selectively impaired but, rather, the production of NO. This is consistent with our previous study using aorta endothe-
lium from dogs with pacing-induced heart failure (24) in which both the mRNA and protein, by Northern and Western analysis, respectively, indicated a 60–80% reduction of ecNOS after heart failure. From those studies we concluded that there is most probably an altered transcriptional regulation of ecNOS after heart failure.

The present studies are supported by measurements of NO production in sieved coronary microvessels from both failing human (9, 10) and canine hearts (33). In these studies, measurements of nitrite production using the Greiss reaction indicated a reduction in NO production in the explanted human hearts at the time of transplantation (9, 10) and a reduction of nitrite production after heart failure but not after 3 wk of pacing in the canine heart (33). In the same study by Zhao et al. (33), we also examined changes in coronary vascular resistance caused by stimulation of the Jarisch reflex in vivo. We found that the reflex, vagal NO-dependent increase in coronary blood flow and the fall in calculated late diastolic coronary resistance (22) were essentially eliminated after the development of severe pacing-induced dilated myopathy (33). We also showed that these reflex-induced responses were NO dependent and that stimulation of the cut end of the vagus nerve in anesthetized dogs resulted in no fall in coronary vascular resistance after the development of severe heart failure (33).

The portion of the vasodilation that was abolished after heart failure in the present study was that which was abolished by an inhibitor of NOS in arterioles from the normal heart. The portion that was blocked by Indo was unchanged after heart failure. There was a small portion of the NO response that remained, as shown by the complete abolition of the response only after the combined inhibition of both NO and prostaglandin biosynthesis. This indicates that there is a small residual NO production and a maintained prostaglandin production after heart failure. The small remaining NO-dependent dilation after heart failure is consistent with a reduction but not complete disappearance of ecNOS mRNA and protein after heart failure in our previous study (24).

It is important to note that flow-induced dilation is inhibited to a greater degree than agonist-induced dilation after the development of heart failure. This is important in two contexts. First, it supports the conclusion that agonist- and flow-mediated dilation may have different transduction mechanisms (18). Second, it has major therapeutic implications in that the maintenance of the responses to bradykinin may indicate that drugs that target the bradykinin receptor may still be effective in eliciting NO production and may be of significant therapeutic value after the development of heart failure. Perhaps the most commonly used drugs in the treatment of heart failure are the angiotensin-converting enzyme (ACE) inhibitors (31, 32). These drugs release NO or, stated more properly, enhance kinin-mediated NO production by inhibiting kininase II and by increasing the half-life of locally formed kinins. In a recent study, we found in sieved coronary microvessels from the human heart (9) that three different ACE inhibitors are capable of releasing NO even after the development of severe heart failure (9). This response was blocked by HOE-140, a bradykinin-2 receptor antagonist, and L-NNA, indicating that the response was kinin and NO dependent. The finding that the bradykinin-mediated dilation persisted after heart failure shows the underlying mechanism of action of ACE inhibitors. In the future, other drugs such as neutral endopeptidase (NEP) inhibitors (enzyme 24.10), alone or in combination with ACE inhibitors, will be used to treat heart failure. These compounds, such as phosphoramidon, stimulate the formation of NO by inhibiting another enzyme that metabolizes kinins. In fact, there is speculation that NEP is the primary mechanism responsible for kinin breakdown when ACE is inhibited. Recent preliminary studies in our laboratory indicate that amiodipine, a second-generation L-type calcium channel antagonist, releases NO by a kinin-dependent mechanism (30). These drugs may all work, at least in part, in the treatment of heart failure by promoting kinin-induced NO production (32). The partial preservation of the bradykinin response in contrast to the shear stress-related dilation may underscore the physiological mechanisms underlying these therapies.

There were some unexpected findings in our study. First, the prostaglandin component of flow-mediated dilation was maintained after heart failure. This is in contrast to our previous finding (24) in aorta indicating that cyclooxygenase-1 was downregulated to a significant degree after heart failure. Another important finding was that there appears to be a heterogeneous decrease in vascular NO production in our model of heart failure. For instance, flow- and agonist-induced dilations of large epicardial coronary artery produced with the use of ultrasonic techniques in conscious dogs (28) were almost completely abolished after the development of decompensated dilated myopathy. The finding that the small coronary microvessel response to agonists remains to a large degree is an important distinc-
tion. The response to ACh may be mediated by an additional mechanism(s), perhaps endothelium-derived hyperpolarizing factor, and may not be useful as an index of NO production in our study.

The results of our studies are in conflict with the results of studies by others indicating that NO production is elevated after the development of severe heart failure. The results of the studies by O’Murchu et al. (19) and Larosa et al. (16) have been interpreted to indicate that NO production is high after the development of heart failure. These data are strikingly similar to our data in hearts after 3 wk of pacing. Because hemodynamic measures were taken in the aforementioned studies in chronically instrumented but anesthetized dogs, it is very likely that the widely recognized cardiac depressant effects of anesthetics, barbiturates in particular, result in artificially elevated ED50 and reduced cardiac output and LV dP/dt (27) in an otherwise compensated state. Thus the heart has all the evidence of failure, but this may be drug induced. To support this contention, a study by Shannon et al. (21) showed that cardiac output was not reduced until 3–4 wk after the initiation of pacing-induced heart failure in awake dogs, whereas O’Murchu et al. (19) found a decrease in cardiac output within 2 wk of the initiation of pacing in chronically instrumented dogs anesthetized for the measurement of hemodynamics. These results also may have been clouded by the use of thermodilution and acutely placed catheters used to measure LV function. A recent study by Khadour et al. (8) showed increased NOS in myocytes of left atria from dogs with pacing-induced heart failure. These authors failed to find changes in NOS gene expression in myocytes from the left ventricle. It should, however, be remembered that only a small fraction of coronary blood flow perfuses the atria, ~1–3% based on a previous study of ours (4), and that this change in gene expression may have little significance in the progression to heart failure. Thus, if we recognize the widely held depressant effects of anesthetics (27) and conclude that other studies overestimate the degree of heart failure, our data from hearts with 3 wk of pacing support the conclusions of the above-mentioned studies.

In summary, our study indicates that NO-dependent dilation is markedly reduced in canine coronary arterioles after pacing-induced heart failure. The somewhat disparate findings of the magnitude of the endothelial dysfunction, determined by using flow and agonists to stimulate NO production, support the conclusion that distinctly different transduction mechanisms exist for flow and agonists and support the use of drugs that release NO through a kinin-dependent mechanism in the treatment of heart failure. The disappearance of the L-NNA-blockable component of the flow-mediated dilation indicates that the primary defect in coronary arterioles in pacing-induced heart failure is in endothelial NO production.

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