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European Journal of Pharmacology 318 (1996) 387–394

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Altered vasodilator response of coronary microvasculature in pacing-induced congestive heart failure

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Received 5 August 1996; revised 4 October 1996; accepted 8 October 1996

Abstract

To characterize vasodilator capacity of small coronary arteries (200–350 μm diameter) in the setting of congestive heart failure, we examined relaxation responses to acetylcholine (10^{-9}–10^{-4} M) and nitroglycerin (10^{-9}–10^{-4} M), in the absence and presence of the nitric oxide precursor, L-arginine (10^{-4} M). Congestive heart failure was reliably induced in dogs by rapid ventricular pacing (250 beats.min^{-1} for 4 weeks). Maximum relaxations (means ± S.E.) to each vasodilator are expressed as a percentage of the relaxation response to papaverine (10^{-4} M). Relaxation responses to the endothelium-dependent relaxing agent, acetylcholine, were not altered at heart failure, or in the presence of L-arginine. Contrary to acetylcholine, relaxations to nitroglycerin were significantly enhanced in heart failure compared to control (83 ± 25% vs. 25 ± 6%, respectively, $P < 0.05$). Although L-arginine, alone, did not cause any vasodilator response in coronary microvessels, it was able to potentiate nitroglycerin relaxations at control (no L-arginine: 25 ± 6% vs. L-arginine: 135 ± 66%). In contrast, at heart failure, L-arginine diminished nitroglycerin relaxations (no L-arginine: 83 ± 25%, vs. L-arginine: 48 ± 15%). These data indicate a unique vasodilator profile in small coronary arteries at heart failure: endothelium-dependent relaxations are unaltered, whereas responses to nitroglycerin are augmented. Addition of the nitric oxide precursor, L-arginine, did not affect acetylcholine relaxation, yet surprisingly had a differential effect in response to nitroglycerin. Moreover, inhibition of nitric oxide synthase with N^{ω}-nitro-L-arginine elicited concentration-dependent constriction in heart failure but not control coronary microvessels. In summary, our study suggests an important role for nitric oxide in vasodilator control of coronary microvessels, which may modify nitrovasodilator therapy in congestive heart failure.

Keywords: Coronary microvessel; Acetylcholine; Nitroglycerin; Heart failure; L-Arginine; Nitric oxide (NO)

1. Introduction

Nitroglycerin is commonly used in the management of ischemic heart disease (Abrams, 1992; Feldman and Conti, 1981), congestive heart failure (Cohn, 1992) and angina (Thadani, 1992), and the effects of nitroglycerin on coronary hemodynamics has been a subject of considerable interest. Nitroglycerin, which is metabolized to nitric oxide in smooth muscle cells, acts by ultimately activating soluble guanylate cyclase in vascular smooth muscle cells, thereby increasing intracellular levels of cyclic guanosine monophosphate (cGMP) and mediating vasodilatation (Shibata et al., 1986; Kukovetz et al., 1979; Axelsson et al., 1981; Gruetter et al., 1981). This pathway for vascular relaxation is also shared by endothelium-dependent vasodilators, such as acetylcholine, which also release nitric oxide by conversion of L-arginine to L-citrulline via nitric oxide synthase (Moncada et al., 1991a).

We have previously shown that endothelium-dependent relaxations in large coronary arteries are enhanced in the setting of pacing-induced congestive heart failure (Larosa et al., 1994; Main et al., 1991). In contrast, others have reported an impairment of endothelium-derived relaxing factor activity at heart failure (Kubo et al., 1991; Treasure et al., 1990; Ontkean et al., 1991; Wang et al., 1994; Kaiser et al., 1989). Such discrepancies may be due to the fact that the latter experiments were performed in vivo and/or in peripheral vascular beds.

Although it is well known that nitroglycerin preferentially dilates large conduit coronary arteries (Winbury et al., 1969; Schnaar and Sparks, 1972; Winbury, 1971; Winbury and Lloyd, 1967), the effects of nitroglycerin in the microcirculation are less clearly defined. Since nitro-

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PII S0014-2999(96)00809-6

glycerin, acetylcholine and endogenous nitric oxide dilate vessels by a common final pathway, they may influence the vasodilator properties of each other (Alheid et al., 1987; Dinerman et al., 1991; Moncada et al., 1991b). Therefore, to further characterize the vasodilator profile of the coronary vascular bed, the present study was undertaken to examine vasodilator responsiveness in small, isolated endothelium-intact coronary arteries (200-350 μm diameter) which were free from metabolic and autoregulatory mechanisms, from dogs with and without pacing-induced congestive heart failure. In particular, the objectives were: firstly, to assess relaxations to both endothelium-dependent and endothelium-independent agents in the setting of congestive heart failure; secondly, to examine the effect of the nitric oxide precursor, L-arginine, on vasodilator responses to acetylcholine and nitroglycerin at control and heart failure; and thirdly to determine the effects on nitric oxide synthase inhibition with N^{ω}-nitro-L-arginine (L-NNA) on coronary microvascular tone.

2. Materials and methods

2.1. Canine model of congestive heart failure

Congestive heart failure was induced by rapid ventricular pacing (250 beats/min for 4 weeks) as previously described (Armstrong et al., 1986). Study groups consisted of male mongrel dogs (18–25 kg) at control (non-paced; $n = 8$) and after pacing-induced congestive heart failure ($n = 15$). Since previous data from our laboratory have indicated that coronary vessels from sham-operated and non-sham-operated (acute) animals behave in a similar manner (Main et al., 1991), control dogs in this study did not have a sham-operation. Dogs in the heart failure group were anaesthetized (sodium thiopental, 25 mg/kg, Abbott Laboratories, Montreal, Québec, Canada), and a pacemaker lead was inserted into the apex of the right ventricle. A pacemaker generator (Medtronics, Mississauga, Ontario, Canada) was implanted into a subcutaneous cervical pocket. Following surgery, dogs were allowed to recover for 7–10 days, and their pacemaker generators were subsequently programmed to deliver 250 beats/min. Dogs with heart failure consistently displayed changes in both hemodynamic and neurohumoral profiles (Armstrong et al., 1986). Clinical manifestations of heart failure were evidenced by an increase in heart size (25%), the presence of pulmonary edema and/or an increase in body mass (10%) (Armstrong et al., 1986). Approval for these studies was obtained from the Animal Care Committee of St. Michael's Hospital and the University of Toronto in accordance with the Animals of Research Act and the guidelines of the Canadian Council on Animal Care.

2.2. Microvessel experiments

At postmortem, dogs were given an overdose of sodium thiopental, the heart was excised and placed on ice. Anterior papillary muscle was then removed from the heart and cut into vertical slices from which small coronary arteries (200–350 μm diameter) were carefully dissected from the subendocardial region using a dissection microscope (Nikon, SMZ-1B). Arteries were obtained from the region of the anterior papillary muscle as, our laboratory has previously reported reduced endocardial to epicardial flow ratios in this region of the heart at heart failure compared to control (Moe et al., 1993). The small coronary arteries were cleaned of connective tissue and fat, and placed in an 8 ml microvessel chamber (Living Systems, Burlington, VT, USA). Preparations were then fastened between two glass cannulae using ophthalmic surgical suture (Ethicon, 10.0, silk). Care was taken not to damage the endothelium of the coronary microvessels, and this was subsequently confirmed by relaxations to acetylcholine. The microvessel chamber was subsequently placed onto a microscope stage (Nikon Inverted Microscope), to which a camera was attached. Images of the vessel could then be visualized on a television monitor and changes in internal lumen diameter were measured with a video dimension analyzer (Living Systems, Burlington, VT, USA) (Halpern et al., 1984), and recorded on a polygraph (Fisher Scientific, Toronto, Ontario, Canada). All preparations were allowed to equilibrate for 1 h in Krebs-Henseleit (Krebs) solution which was delivered to the bath via a peristaltic pump (Buchler Instruments, Fort Lee, NJ, USA). The maximum constriction to KCl (100 mM) occurred at a distending pressure of 10 mmHg for both control and heart failure preparations (pilot experiments). Vessels were therefore pressurized to 10 mmHg in a no-flow state for all subsequent experiments. The Krebs solution was of the following mM composition: glucose, 10; $NaHCO_3$, 25; KCl, 5.6; NaCl, 120; $MgSO_4$, 1.2; NaH_2PO_4, 1.2; and $CaCl_2$, 2.5. In addition, indomethacin (5 μM), desipramine (1 μM) and propranolol (5 μM) were incorporated into the Krebs to inhibit endogenous prostanoid production, and to eliminate any modification due to sympathetic activation induced by any of the exogenous stimuli. Preparations were constantly bubbled with a gas mixture of 95% oxygen and 5% carbon dioxide, and bath temperature was maintained at 37°C via a circulating water pump (Haake D2, Fisher Scientific).

2.3. Agonist concentration-effect studies

Following equilibration, the resting internal basal diameter for each artery was noted, and a concentration-effect curve to KCl (5–100 mM) was generated. Constriction to KCl is expressed as percentage change from baseline internal diameter, with total occlusion representing 100% constriction. From the KCl concentration-effect curve, an EC_{50} value for KCl was determined and subsequently used to preconstrict arteries prior to vasodilator administration. KCl was used to preconstrict vessels as it does not affect the degree of vasodilatation in coronary arteries (Angus and Brazenor, 1983; Ito et al., 1980), and it does not contribute to endothelium-dependent relaxations as do other

vasoconstrictors such as noradrenaline (Larosa et al., 1994). Arteries were washed until diameter returned to baseline value, and KCl (EC_{50} value) added to the bath. Since tolerance to nitroglycerin can cause cross-tachyphylaxis of other vasodilating agents (Parker and Parker, 1992), nitroglycerin was administered after acetylcholine. Following plateau of the constrictor response, a cumulative concentration-effect curve was constructed to the endothelium-dependent agent, acetylcholine (10^{-9}–10^{-4} M) as a functional test for endothelial integrity (Furchgott, 1984). Preparations were then washed until baseline diameter was again reached. This time, after preconstriction with KCl (EC_{50} dose), arteries were challenged with the endothelium-independent vasodilator, nitroglycerin (10^{-9}–10^{-4} M). Exposure of the arterial rings to different relaxants was separated by at least 60 min, during which time preparations were continuously washed with Krebs. All concentration-effect curves to the vasodilators were repeated after incubation with the active or inactive nitric oxide precursor, L-arginine and D-arginine, respectively (20 min, 10^{-4} M) (Sakuma et al., 1988). Following a washout period, coronary microvessels were challenged with the nitric oxide synthase inhibitor, L-NNA, to assess the contribution of nitric oxide on basal resting diameter. At the end of the experiment, arteries were again preconstricted with KCl (EC_{50} value) and challenged with the non-nitroso, cGMP-independent vasodilator, papaverine (10^{-4} M), to determine maximal relaxation response (Gharaibeh and Gross, 1984; Shimizu et al., 1980) in control and heart failure preparations. Relaxations induced by acetylcholine and nitroglycerin were expressed relative to the maximum relaxation produced by the papaverine challenge (Gharaibeh and Gross, 1984).

2.4. Drugs and solutions

The following drugs were used: acetylcholine iodide, desipramine hydrochloride, indomethacin, L-arginine hydrochloride, and papaverine (from Sigma, St. Louis, MO, USA); and nitroglycerin from DuPont Canada (Scarborough, Ontario, Canada). L-Propranolol hydrochloride was donated from Ayerst Laboratories (New York, NY, USA), and KCl was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Drug dilutions were prepared fresh daily, and were kept on ice during the experiment. All drug concentrations are expressed as final molar concentration present in the bath. The vehicle for nitroglycerin contained 0.3 ml alcohol and 0.3 ml propylene glycol, and 9.4 ml distilled water. This stock solution was subsequently diluted in distilled water as it did not affect relaxation responses, as addressed in a previous report from our laboratory (Forster et al., 1990).

2.5. Data analysis

All data are expressed as means ± S.E., with n indicating the number of dogs used (one preparation/dog was studied). Magnitude of vasoconstriction and vasodilatation are represented as percentage change in baseline internal diameter measured at the beginning of each concentration-effect curve. Estimates of the effective concentration of agonist producing 50% of the maximum response (EC_{50}) and the maximum percent relaxation were determined using a curvfit program. The data processing package fits the points of individual concentration-effect curves to a sigmoidal function which is derived from the logistic: $Y = \{(a - d)/[1 + (X/c)^b]\} + d$, where Y is the response, X is the arithmetic dose, a is the response when $X = 0$, d is the response for an infinite dose (i.e., maximum response), c is the EC_{50}, and b is the slope factor (Parker and Waud, 1971). Individual EC_{50} values are expressed as the geometric mean with 95% confidence limits. Maximum relaxation responses to acetylcholine and nitroglycerin are expressed as a percentage of the maximum vasodilator response to papaverine (Gharaibeh and Gross, 1984). Statistical analysis of data was performed using Student's t-test for paired and unpaired data and a Bonferroni correction was applied for multiple comparisons, where appropriate (Wallenstein et al., 1980). A P value of < 0.05 was considered significant. Difference between concentration-effect curves was assessed by area above the curve as calculated by the trapezoidal rule (Rowland and Tozer, 1980), and a χ^2-test statistic was used to determine significant differences. The non-parametric Mann-Whitney test was used to compare EC_{50} values.

3. Results

The hemodynamic profile of control and heart failure dogs is shown in Table 1. Heart failure dogs displayed a significant reduction in cardiac output, and marked elevation in heart rate and cardiac filling pressures ($P < 0.05$ vs. control).

The mean resting internal diameter was similar in control (322 ± 31 μm) and heart failure (302 ± 23 μm) coronary microvessels (NS). Concentration-effect curves to the vasoconstrictor, KCl, in small coronary arteries from control and heart failure groups are shown in Fig. 1, and generated EC_{50} values of 29.5 mM (24.7–35.2) and 41.7 mM (33.6–51.7), respectively ($P < 0.05$). It can also be seen that contractions to KCl were not different between control and heart failure preparations (Fig. 1), reaching maxima of 33.0 ± 5.1 and 31.9% ± 2.5%, respectively.

In vessels preconstricted with KCl, papaverine elicited similar magnitudes of relaxation in control and heart failure groups. Maximum percentage relaxations to papaverine were 39 ± 12 and 36 ± 10%, for control and heart failure coronary microvessels, respectively. Since papaverine-induced relaxations were comparable between control and heart failure groups, relaxations to the other vasodilators at control and heart failure were expressed relative to the papaverine response.

Table 1
Hemodynamic measurements in control and heart failure dogs

	Control	Heart failure
Cardiac output (l/min)	3.5 ± 0.3	1.9 ± 0.1 [a]
Heart rate (bpm)	81.0 ± 6.2	134.7 ± 6.1 [a]
Mean aortic pressure (mmHg)	110.0 ± 8.4	97.7 ± 2.4
Pulmonary artery pressure (mmHg)	16.9 ± 1.1	38.9 ± 2.0 [a]
Pulmonary capillary wedge pressure (mmHg)	8.2 ± 0.5	27.8 ± 1.9 [a]
Right atrial pressure (mmHg)	6.6 ± 0.4	13.7 ± 0.7 [a]
Left ventricular end diastolic pressure (mmHg)	9.9 ± 1.4	36.3 ± 2.4 [a]

Hemodynamic parameters (means ± S.E.) in dogs at control and following pacing-induced congestive heart failure (4 weeks pacing). The following measurements were taken: cardiac output, heart rate, mean aortic pressure, pulmonary artery pressure, pulmonary capillary wedge pressure, right atrial pressure, and left ventricular end diastolic pressure. Hemodynamic evaluation was performed in normal sinus rhythm. Units for each parameter are indicated in parentheses. Data are derived from 8 control dogs and 14 heart failure dogs. [a] $P < 0.05$ vs. control.

3.1. Relaxation response to acetylcholine in the absence and presence of L-arginine

Concentration-effect curves to acetylcholine, before and after treatment with L-arginine (10^{-4} M), are shown in Fig. 2 for control and heart failure coronary arteries. Mean magnitude of KCl-induced constriction (approximate EC_{50} dose), prior to the administration of acetylcholine, was identical for control and heart failure arteries (as percentage of KCl maxima: 56.9% and 61.0%, respectively). Maximum relaxation to acetylcholine (expressed as a percentage of maximum papaverine response) reached 104 ± 51% at control, and 124 ± 29% at heart failure (NS).

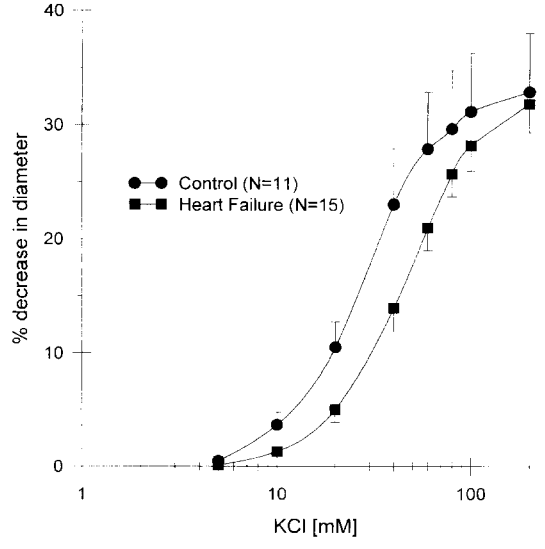

Fig. 1. Concentration-effect curves to KCl. Vasoconstrictor responses (percentage decrease from resting internal diameter) to KCl in canine coronary microvessels (200–400 μm diameter) at control and following experimental heart failure. Values are means ± S.E., and n indicates number of dogs.

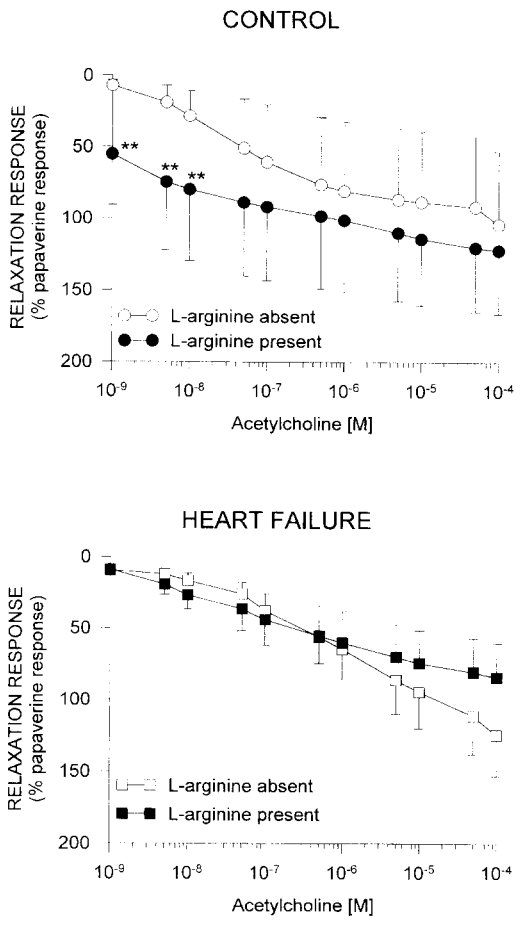

Fig. 2. Concentration-effect curves to acetylcholine. Relaxation responses to acetylcholine in endothelium-intact small canine coronary arteries at control (top panel) and in heart failure (bottom panel). Arteries were preconstricted with KCl (EC_{50} dose) prior to the administration of acetylcholine. Responses to acetylcholine were obtained both in the absence (open symbols) and in the presence (closed symbols) of the nitric oxide precursor, L-arginine (10^{-4} M). Relaxations are means ± S.E. and are expressed as a percentage of the relaxation response to papaverine (10^{-4} M). Data are from $n = 5$–16 preparations. ** $P < 0.05$, vs. no L-arginine.

Addition of L-arginine did not significantly alter these acetylcholine-mediated relaxations at control or at heart failure (maximum relaxations were 122 ± 44% and 87 ± 24%, respectively). However, L-arginine did significantly augment relaxations to low concentrations of acetylcholine (10^{-9}–10^{-8} M) in control preparations ($P < 0.05$) (Fig. 2). This effect was absent in heart failure coronary microvessels. Sensitivity of small coronary arteries to acetylcholine, as indicated by EC_{50} values, is shown in Table 2 for both control and heart failure groups. No difference was seen in the potency of acetylcholine between control and heart failure coronary microvessels.

Areas above the curve for acetylcholine (Table 3) in the absence of L-arginine were not different between control and heart failure groups. In the presence of L-arginine, however, area under the curve in the heart failure group was reduced compared to control ($P < 0.005$).

Table 2
EC_{50} values to acetylcholine and nitroglycerin in small canine coronary arteries at control and in heart failure

	Control	Heart failure
Acetylcholine	2.0×10^{-7}	5.2×10^{-7}
	(0.3–11.9)	(2.1–13.0)
	($n=8$)	($n=15$)
Acetylcholine + L-arginine	3.2×10^{-8}	1.9×10^{-7}
	(0.1–87.2)	(0.3–13.3)
	($n=5$)	($n=9$)
Nitroglycerin	3.3×10^{-7}	1.3×10^{-7}
	(0.1–85.4)	(0.2–9.3)
	($n=3$)	($n=12$)
Nitroglycerin + L-arginine	1.1×10^{-7}	2.4×10^{-8}
	(0–127.2)	(0.4–12.7)
	($n=3$)	($n=5$)

EC_{50} values (M units, geometric means with 95% confidence limits) in response to acetylcholine and nitroglycerin in the absence and presence of L-arginine (10^{-4} M) from control and heart failure canine coronary arteries. Mean internal diameter of small coronary arteries (mean ± S.E.) was 322 ± 31 μm and 302 ± 23 μm in control and heart failure groups, respectively. n indicates number of preparations.

3.2. Relaxation responses to nitroglycerin in the absence and presence of L-arginine

Fig. 3 shows relaxation responses to nitroglycerin, in control and heart failure arteries. Mean percentage decrease in internal diameter to KCl (EC_{50} dose) was not significantly different. Maximum relaxation responses to nitroglycerin (as percent of papaverine response) were significantly greater in heart failure coronary arteries compared to control (83 ± 25% vs. 25 ± 6%, $P < 0.05$).

Pretreatment with L-arginine enhanced nitroglycerin maximum response at control (134 ± 66% vs. 25 ± 6%), yet attenuated nitroglycerin maximum relaxation at heart failure (48 ± 15% vs. 83 ± 25%). Interestingly, L-arginine potentiated relaxation responses at all doses of nitroglycerin in control, but not heart failure coronary microvessels. Sensitivity of small coronary arteries to nitroglycerin was not significantly different at control and in heart failure, as indicated by EC_{50} values (Table 2).

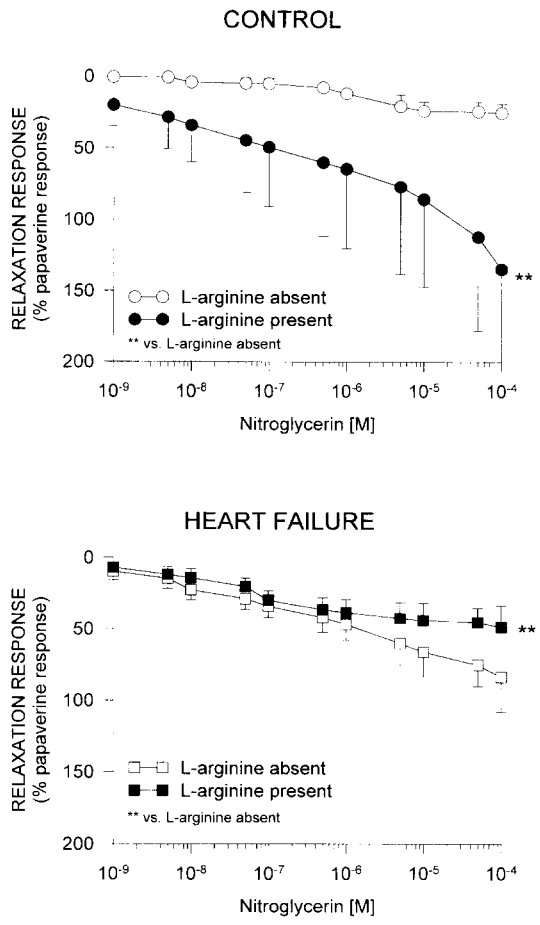

Fig. 3. Concentration-effect curves to nitroglycerin. Relaxation responses to nitroglycerin in endothelium-intact small canine coronary arteries at control (top panel) and in heart failure (bottom panel). Arteries were preconstricted with KCl (EC_{50} dose) prior to the administration of nitroglycerin. Responses to nitroglycerin were obtained both in the absence (open symbols) and in the presence (closed symbols) of the nitric oxide precursor, L-arginine (10^{-4} M). Relaxations are means ± S.E. and are expressed as a percentage of the relaxation response to papaverine (10^{-4} M). Data are from $n = 3$–12 preparations. ** $P < 0.05$ vs. no L-arginine.

Table 3
Areas above concentration-effect curves in response to acetylcholine and nitroglycerin

	Control	Heart failure
I. Acetylcholine		
Acetylcholine	318.5	273.8
Acetylcholine + L-arginine	477.0	248.9 [a]
II. Nitroglycerin		
Nitroglycerin	56.0	209.8 [a]
Nitroglycerin + L-arginine	310.6 [b]	151.5 [a]

Areas above the concentration-effect curves (AAC, in % log M units) for acetylcholine (from Fig. 3) and nitroglycerin (from Fig. 4) are shown. Area above each curve was calculated from the trapezoidal rule (Rowland and Tozer, 1980): $AAC_{0\text{-}C_n} = (R_1 + R_2)/2[C_2 - C_1] + (R_2 + R_3)/2[C_3 - C_2] + (R_{n-1} + R_n)/2[C_n] - C_{n-1}]$, where C represents drug concentration in log M units, and R represents relaxation response (as percentage of papaverine). $P < 0.005$, [a] vs. control; [b] vs. no L-arginine.

Areas above the curve for nitroglycerin (Table 3) in the absence of L-arginine showed a four-fold increase at heart failure compared to control ($P < 0.005$). Addition of L-arginine increased the area above the curve for nitroglycerin at control by six-fold ($P < 0.005$). Moreover, in the presence of L-arginine, the area above the curve at heart failure was reduced compared to control to a similar degree as that seen with acetylcholine ($P < 0.005$).

D-Arginine was found not to affect nitroglycerin relaxation responses of coronary microvessels from control and heart failure groups.

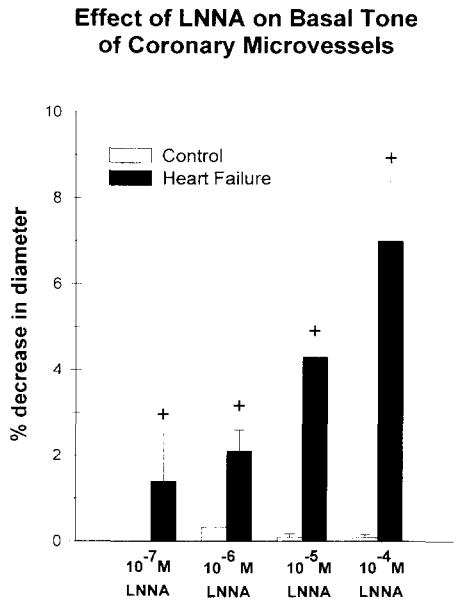

Fig. 4. Effect of nitric oxide synthase inhibition on resting baseline diameter in canine coronary microvessels. The effect of nitric oxide synthase inhibition with N^{ω}-nitro-L-arginine (L-NNA: 10^{-7}M, 10^{-6}M, 10^{-5}M, and 10^{-4}M) on baseline diameter of small coronary arteries is shown. Mean internal resting diameters were 322 ± 31 μm and 302 ± 23 μm for control and heart failure preparations, respectively. Responses are expressed as a percentage reduction in baseline diameter (means ± S.E.). Data are derived from $n = 4$ control and $n = 4$ heart failure dogs. + $P < 0.05$ vs. control.

3.3. Effect of nitric oxide synthase inhibition

At control, basal tone of coronary microvessels was minimally affected by increasing concentrations of the nitric oxide synthase inhibitor, N^{ω}-nitro-L-arginine (L-NNA) [Fig. 4]. Resting internal diameter in these small coronary arteries at control was reduced by 0.33 ± 0, 0.09 ± 0.09, and 0.08 ± 0.08% in response to 10^{-6}M L-NNA, 10^{-5}M L-NNA, and 10^{-4}M L-NNA, respectively. In contrast, resting internal diameter of heart failure coronary microvessels was decreased dose dependently by L-NNA [Fig. 4]. The percentage reduction in baseline diameter of these heart failure vessels to 10^{-7}M, 10^{-6}M, 10^{-5}M, and 10^{-4}M L-NNA was: 1.4 ± 1.1, 2.1 ± 0.5, 4.3 ± 0, and 7.0 ± 2.4%, respectively ($P < 0.05$ vs. control).

4. Discussion

The current study reveals a preservation of endothelium-dependent relaxations to acetylcholine in coronary microvessels (200–350 μm) following pacing-induced congestive heart failure. In contrast, nitroglycerin-mediated relaxations were significantly augmented in heart failure. Addition of L-arginine did not modify acetylcholine-induced relaxations at either control or heart failure, yet it potentiated relaxations to nitroglycerin at control and attenuated them at heart failure. Our finding that nitroglycerin was able to elicit vasodilatation in these vessels at control is consistent with previous studies (Sellke et al., 1991, 1990; Kurz et al., 1991; Kanatsuka et al., 1992).

Despite unaltered endothelium-dependent relaxations at heart failure, there was a selective enhancement of vasodilatation in response to nitroglycerin. The coronary microvessels showed no difference in the magnitude of response to KCl at heart failure, suggesting that abnormal contractile function is unlikely to explain these data. Therefore, possible mechanisms for this increase in relaxation response may involve: more efficient metabolism of nitroglycerin, increased cGMP production, more efficient removal of intracellular Ca^{2+}, increased sensitivity of vascular smooth muscle cells to nitric oxide, and/ or a synergistic effect of elevated basal nitric oxide production in heart failure.

It is possible that smooth muscle cells may have a greater capacity to metabolize nitroglycerin in heart failure. However, the observation that relaxations to nitroglycerin in coronary microvessels > 100 μm in diameter were not affected by addition of the sulfhydryl-donating compound, L-cysteine (Sellke et al., 1991) is against such a mechanism.

L-arginine did not modulate acetylcholine relaxations at heart failure. Enhanced basal production of nitric oxide in coronary microvessels at heart failure has been demonstrated by the concentration-dependent vasoconstriction response to L-NNA. Despite this, endothelial nitric oxide synthase may become downregulated by exposure to endogenous nitric oxide itself (Buga et al., 1993; Rogers and Ignarro, 1992). Such a downregulation of endothelial nitric oxide synthase may act to counterbalance any potential effects of exogenous acetylcholine.

Long-term exposure of vascular smooth muscle to nitric oxide may also downregulate soluble guanylate cyclase activity, causing impaired relaxation to nitrovasodilators (Moncada et al., 1991b). The attenuating effect of L-arginine on nitroglycerin relaxations at heart failure supports this hypothesis. Desensitization of soluble guanylate cyclase is interesting as nitric oxide also causes vasorelaxation by stimulating the same enzyme (Ignarro, 1989; Moncada et al., 1991a; Rapoport et al., 1985). In agreement with this hypothesis, the inhibition of vascular nitric oxide synthesis (by endothelial denudation or L-arginine analogs) sensitized vasorelaxation to nitrovasodilators (Moncada et al., 1991b), and enhanced nitroglycerin relaxations (Busse et al., 1989; Dinerman et al., 1991; Kukovetz and Holzmann, 1989).

Schwartz et al. (1994) have reported a synergistic relationship between nitroglycerin and acetylcholine in patients with congestive heart failure. In agreement with this hypothesis, exogenous L-arginine was able to potentiate nitroglycerin relaxations of coronary microvessels at control. Moreover, recent data showed that L-arginine en-

hanced nitroglycerin effects, such that nitroglycerin actions were dependent on L-arginine (Abou-Mohamed et al., 1996). However, in vivo animal studies have shown an inhibitory interaction between endothelium-derived nitric oxide and nitroglycerin, suggesting competition for activation of processes leading to smooth muscle relaxation. This distinct response suggests a heterogeneous effect of L-arginine on coronary microvessels in normal and diseased states. The mechanism underlying this differential effect of L-arginine is unknown, but may occur as a result of a downregulation of nitric oxide synthase, in response to elevated basal production of nitric oxide at heart failure. Moreover, L-arginine did potentiate relaxations by acetylcholine (low doses) at control, but had no effect on low doses of acetylcholine at heart failure. This is in keeping with impaired nitric oxide synthesis reported in heart failure (Seyedi et al., 1992). Although it can be argued that basal tone may be affected by L-arginine, it had no effect on baseline diameter in control and heart failure arteries. Interestingly, area under the curve in the presence of L-arginine at heart failure was half of that at control, for both acetylcholine and nitroglycerin. This effect may involve changes in the levels of cyclic GMP (i.e., beyond the level of soluble guanylate cyclase), which have not yet been determined. Alternatively, there may be impaired action of the nitric oxide/L-arginine pathway on processes occurring after the synthesis of cyclic GMP, such as protein phosphorylation, Ca^{2-} mobilization or Ca^{2+} sensitivity as suggested by Matsumoto et al. (1993).

In summary, we have demonstrated that heart failure coronary microvessels (200–350 μm) elicit unaltered endothelium-dependent relaxations to acetylcholine compared to control. Endothelium-independent vasodilatation in response to nitroglycerin, on the other hand, was enhanced in heart failure coronary microvessels. This increased vasodilator effect of nitroglycerin in heart failure is relevant when we consider that stimulated or mature collaterals are often > 200–300 μm in diameter (Schaper, 1971). This novel heterogeneous response may be a form of vascular compensation in light of the impaired venodilatation and/or increased vascular resistance to nitroglycerin therapy reported in patients with severe heart failure (Petrasko et al., 1991; Armstrong et al., 1980). Moreover, the novel effect of L-arginine on nitroglycerin has important clinical implications, as it addresses a potential interaction between nitric oxide and nitrovasodilators in the coronary circulation at heart failure. It has been reported that during decreased perfusion pressure, recruitable vasodilatation in response to nitroglycerin is due to dilatation of microvessels > 200 μm (Kanatsuka et al., 1992). The interaction of L-arginine with nitroglycerin, may be important since basal nitric oxide is enhanced in heart failure, and chronic nitroglycerin therapy may actually produce tolerance to the beneficial vasodilatation effect in these patients. In light of these observations, an alternative therapeutic intervention may be aimed at paradoxically reducing endogenous nitric oxide activity in patients who have developed nitrate tolerance. Ultimately, nitroglycerin's beneficial vasodilator effect may be selectively enhanced in the absence of functioning endothelium (Bassenge and Heusch, 1990).

Acknowledgements

The authors would like to thank Medtronics, Canada for their generous supply of pacemakers, and are grateful to DuPont Canada and Ayerst for their generous supply of nitroglycerin and *l*-propranolol, respectively. This research was supported by the Heart and Stroke Foundation of Ontario. G.L. is a recipient of an Ontario Graduate Scholarship.

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