Inhibitory Role of the Coronary Arterial Endothelium to α-Adrenergic Stimulation in Experimental Heart Failure

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The role of the endothelium in regulating coronary α-adrenergic tone was evaluated in isolated coronary arterial rings from dogs with and without pacing-induced congestive heart failure (CHF). The maximal contractile response to methoxamine was attenuated approximately 43% (p<0.05) in both intact and denuded CHF rings compared with control. Conversely, norepinephrine-induced contractions were diminished 58% in intact CHF vessels and 39% in denuded CHF vessels (p<0.05). Denudation did not alter responses to methoxamine but significantly (p<0.05) augmented the tension generated by norepinephrine in both control (1.7-fold) and CHF (2.4-fold) arteries. In both intact control and CHF coronary arteries, norepinephrine elicited rapid, transient relaxations that preceded slow, sustained contractions; the initial relaxation phase was endothelium dependent, because denudation eliminated the response. Relaxations to the selective α₁-adrenoceptor agonist BHT 920 were also dependent on the presence of an endothelium. At peak CHF, endothelium-dependent relaxations to norepinephrine and BHT 920 were enhanced, whereas relaxations to nitroglycerin and acetylcholine were unaltered. The data suggest that α-adrenergic tone in canine coronary arteries is diminished by pacing-induced CHF because of a decrease in α₁-adrenoceptor–mediated constriction and an enhanced capacity of the endothelium to antagonize the direct vascular smooth muscle response of norepinephrine through endothelium-dependent, α₁-adrenoceptor–mediated relaxations. (Circulation Research 1991;68:940–946)

Adrenergic responses within the coronary vasculature are complex because of the existence of a mixed population of α- and β-adrenoceptors. Also, coronary α-adrenergic receptors mediate vasoconstriction. However, both circulating and neurally released catecholamines exert a tonic vasodilatory effect on epicardial vessels via both β₁- and β₂-adrenoceptor activation. Furthermore, α₂-adrenoceptors, located on the endothelium, may mediate the release of endothelium-derived relaxing factor (EDRF), resulting in direct relaxation or inhibition of contraction. Thus, α-adrenergic responses in large canine coronary arteries are a combination of both vasoconstrictor (α₁-adrenoceptor) and vasodilator (α₂-adrenoceptor) mechanisms.

The effect of congestive heart failure (CHF) on the adrenergic control of coronary arterial tone remains unknown. CHF is associated with an array of peripheral neurohumoral adjustments, including activation of the sympathetic nervous system. Rapid ventricular pacing in the dog produces hemodynamic and neurohumoral perturbations similar to those found in patients with CHF, including a rise in plasma norepinephrine. Indeed, tachycardia-induced heart failure has been described as a specific clinical syndrome in humans. Preliminary in vitro studies indicate a diminished contractile responsiveness to α-adrenergic stimulation in endothelium-intact coronary arteries from dogs with pacing-induced heart failure. Activation of the sympathetic nervous system may modify the integrated response within coronary vessels between α₁-adrenoceptor–mediated vascular smooth muscle constriction and α₂-adrenoceptor–mediated, endothelium-dependent vasodilation. The physiological or pathophysiological role of EDRF within the coronary vasculature in CHF, however, has not been addressed. Consequently, the objective of the present study was to assess the influence of the endothelium on α-adrenoceptor–mediated contractions in epicardial coronary arteries from dogs with pacing-induced heart failure.

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Materials and Methods
Canine Model of Congestive Heart Failure

CHF was produced in eight male mongrel dogs (18–25 kg) by rapid ventricular pacing. Approval for the studies was obtained from the Animal Care Committee of St. Michael’s Hospital in accordance with the Animals of Research Act and the guidelines of the Canadian Council on Animal Care. Briefly, a pacemaker generator was inserted into a cervical pocket, and a unipolar pacemaker lead was positioned in the right ventricle under general anesthesia. After 5–7 days of recovery, the pulse generator was programmed to deliver 250 beats/min asynchronously. Dogs were examined biweekly for signs of dyspnea and pulmonary edema, as well as the presence of ascites. Animals having fulfilled previously defined criteria for severe heart failure, that is, a 10% increase in body weight or a 25% increase in heart size accompanied by the presence of pulmonary edema on a chest radiogram, were killed with an overdose of sodium thiopental.

Three animals that were implanted with inactive pacemakers were followed similarly for 4–6 weeks, and three additional dogs were killed acutely. Tissue bath data from these two sources were comparable and therefore were combined and designated as the control group.

Organ Bath Experiments

The heart was excised and quickly placed in 4°C Krebs-Henseleit solution (Krebs) of the following millimolar composition: NaCl 120, KCl 5.6, CaCl₂ 2.5, MgSO₄ 1.2, NaH₂PO₄ 1.2, NaHCO₃ 25, and glucose 10. Approximately 3–4 cm of left anterior descending and left circumflex artery were dissected from the point of bifurcation from the left main coronary artery and placed in Krebs at room temperature. The blood vessels were cleaned of connective tissue without disrupting the luminal surface and then were used either immediately or refrigerated overnight in Krebs. Our laboratory and others have shown that vascular preparations do not lose their reactivity when stored at 4°C. Each artery was cut into 5-mm rings. The endothelium was disrupted in alternate rings by inserting the tip of a fine forceps into the lumen and gently rolling the preparation back and forth over filter paper moistened with Krebs. Vascular rings were studied in parallel in organ chambers containing 10 ml of Krebs gassed with 95% O₂–5% CO₂ (37°C, pH 7.4). Propanolol (5 μM), indomethacin (5 μM), desipramine (1 μM), and hydrocortisone (10 μM) were present in all experiments to antagonize β-adrenoceptors, inhibit endogenous prostanoïd production, and block neuronal uptake and extraneuronal uptake, respectively. Each ring was suspended by means of two platinum and nickel L-shaped hooks passed through the lumen. The lower hook was anchored to a metal tissue holder inside the organ bath, and the other was connected with 4.0 surgical suture (Ethicon, silk) to a force transducer (model FT03c, Grass Instrument Co., Quincy, Mass.). Changes in isometric tension were recorded and amplified using a Grass polygraph (model 7D).

The length–active tension relation in the left anterior descending and circumflex coronary arteries from both control and CHF dogs was examined in preliminary experiments, and the optimal resting tension was determined as 4 and 6 g, respectively. No difference was observed between vessels from the control and CHF groups.

Agonist Concentration–Effect Studies

Cumulative concentration–effect curves were constructed in intact and denuded rings after a 60–90-minute equilibration period. A minimum of five different agonists was administered to each individual preparation. The sequence of drug exposure was randomized, and consecutive curves were separated by a 60-minute wash period.

Adrenergic contractile activity was assessed using the selective α₁-agonist methoxamine (0.01–100 μM) and the mixed α₁-agonist norepinephrine (0.01–10 μM). To determine the role of endothelial adrenoceptor function in CHF, the selective α₂-agonist BHT 920 (0.1–100 μM) was administered to rings precontracted with 10 mM KCl, an approximate EC₃₀ dose derived from preliminary experiments.

General vessel responsiveness was assessed with KCl (20–80 mM), whereas relaxations elicited by acetylcholine (0.1–10 μM) (in preparations precontracted with either 20 mM KCl or 2.0 μM prostaglandin [PG] F₂ₐ) were used to ascertain the integrity of the endothelium. The capacity of the arterial segments to relax to an endothelin-independent agent, nitroglycerin (1.0 nM–10 μM), was also evaluated (all rings precontracted with 2 μM PGF₂α).

Drugs and Solutions

The following drugs were used: acetylcholine iodide (Sigma Chemical Co., St. Louis), BHT 920 (Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, Conn.), desipramine hydrochloride (Sigma), hydrocortisone (Sigma), indomethacin (Sigma), methoxamine hydrochloride (Burroughs Wellcome Co., Research Triangle Park, N.C.), nitroglycerin, 1-norepinephrine bitartrate (Sigma), propanolol hydrochloride (Ayerst Laboratories, New York), and PGF₂α (The Upjohn Co., Kalamazoo, Mich.). Indomethacin and hydrocortisone were dissolved in sodium bicarbonate and ethanol, respectively, before being added to the Krebs solution. Catecholamine stock solutions contained 0.2% ascorbic acid as an antioxidant. Baseline tension was unaffected by the drug vehicles in the concentrations used. PGF₂α stock solutions (2.0 mM) were refrigerated for up to 3 months, whereas all other drugs were prepared fresh daily. Dilutions were made in Krebs or distilled water and kept on ice during the experiment. Concentrations are expressed as the molar concentration that was present in the bath.
Data Analysis

Contractions are expressed as mean±SEM for the number (n) of rings. To eliminate differences due to variability in muscle mass, all responses were normalized for cross-sectional area (grams per square millimeter).\textsuperscript{14} Relaxations (mean±SEM) are defined as the percent change in tension induced by the contractile agent; that is, 100% represents relaxation to baseline tension. EC\textsubscript{50} and IC\textsubscript{50} values (concentrations producing 50% of the maximal contraction or relaxation, respectively) are expressed as geometric means with 95% confidence intervals and were derived from individual EC/IC\textsubscript{50} estimates calculated by a computer program that fit the points of individual concentration–effect curves to a sigmoidal relation. Statistical comparisons were performed with Student’s t tests for unpaired observations (two-tailed) using a Bonferroni correction for multiple comparisons.\textsuperscript{15} A $\chi^2$ test was used to assess differences between independent proportions. A value of $p<0.05$ was considered significant.

Results

Removal of the endothelium did not affect the increases in tension produced by the selective $\alpha_1$-agonist methoxamine in either control or CHF segments (Figure 1A). The maximal methoxamine response in CHF arteries, both with and without an endothelium, was significantly lower than that obtained in the corresponding control segments (58% of control in intact CHF segments [0.82±0.12 g/mm\textsuperscript{2} versus 1.42±0.17 g/mm\textsuperscript{2}; $p<0.05$] and 56% of control in denuded CHF preparations [0.98±0.14 g/mm\textsuperscript{2} versus 1.76±0.19 g/mm\textsuperscript{2}; $p<0.05$]). The sensitivity of the coronary arteries to methoxamine was not altered by denudation and was similar in control and CHF segments (9.5 μM [6.8–11.6] control/intact versus 10.6 μM [8.8–12.8] control/denuded and 8.9 μM [6.9–11.6] CHF/intact versus 9.3 μM [6.9–12.5] CHF/denuded).

Contractile responses to norepinephrine were significantly enhanced, in both control and CHF rings, by the mechanical disruption of the endothelium (Figure 1B); the maximal tension generated in control vessels was increased 1.7-fold (1.49±0.17 g/mm\textsuperscript{2} versus 2.47±0.25 g/mm\textsuperscript{2}; $p<0.05$, intact versus denuded) and in CHF vessels was increased 2.4-fold (0.62±0.09 g/mm\textsuperscript{2} versus 1.51±0.14 g/mm\textsuperscript{2}; $p<0.05$, intact versus denuded).

Independent of the endothelium, CHF arteries pulled significantly less tension to norepinephrine as compared with control arteries (Figure 1B). The maximal response to norepinephrine in denuded CHF segments was 61% of the denuded control response, whereas intact CHF vessels produced only 42% of the intact control response to norepinephrine. The maximal contractile force developed in denuded CHF rings (1.51±0.14 g/mm\textsuperscript{2}) was similar to that evoked by the agonist in intact control rings (1.49±0.17 g/mm\textsuperscript{2}). The variable nature of the norepinephrine response (see below) precluded EC\textsubscript{50} determination.

In individual rings with an intact endothelium, norepinephrine evoked a dose-related biphasic response that was characterized by an initial rapid decrease in tension, with a peak response occurring in less than 30 seconds, followed by a slow, sustained contraction, with a peak response occurring over minutes (Figures 2A and 2B). Low concentrations of norepinephrine (<0.1 μM) generally produced contractions only. Differences were noted in the proportion of control and CHF preparations that responded biphasically to norepinephrine; endothelium-dependent relaxations occurred in only 17 of 37 control arteries examined compared with 36 of 39 CHF arteries studied ($p<0.05$; $\chi^2$). In coronary arteries that displayed biphasic responses, the concentration of norepinephrine at which endothelium-dependent relaxations first appeared was 10-fold lower in CHF arteries compared with control (Figure 3). The magnitude of the relaxation for any given dose of norepi-
norepinephrine also was significantly greater in CHF preparations, relative to control.

Vessels with a functional intimal layer never evoked biphasic relaxations and contractions to the \( \alpha_1 \)-agonist methoxamine. Similarly, biphasic norepinephrine responses (to individual dosage increments) were never observed in control or CHF rings whose intimal surface had been removed intentionally (Figure 2C). Concentrations of norepinephrine up to 10 \( \mu \text{M} \) invariably produced contractions in denuded preparations. Doses of more than 10 \( \mu \text{M} \) elicited relaxations that were slow to develop and were sustained over time in both intact and denuded rings.

Figure 4 shows that intact control segments responded to BHT 920 with small dose-dependent relaxations. Conversely, denuded control rings contracted to the \( \alpha_1 \)-agonist over the entire range of concentrations examined. The effects of BHT 920 in intact and denuded CHF preparations were similar but more profound. Relative to control, maximal contractions to BHT 920 were depressed in denuded CHF arteries, whereas BHT 920–induced relaxations were enhanced in intact CHF vessels.

The maximal force generated in response to KCl in intact CHF vessels (4.3±0.3 g/mm\(^2\)) was similar to that in intact control vessels (4.9±0.4 g/mm\(^2\)), but denuded CHF rings (3.9±0.2 g/mm\(^2\)) produced 25% less tension than did denuded control rings (5.1±0.4 g/mm\(^2\)) \((p<0.05)\). In both control and CHF rings, removal of the endothelium did not affect contractions due to KCl.

**Figure 2.** Representative tracings of control and heart failure arteries with and without endothelium in response to norepinephrine. Panel A: Intact artery from control dog; panel B: intact artery from heart failure dog; panel C: denuded artery from heart failure dog. Concentrations are micromolar. The vertical bar on the scale represents grams of tension. All recordings begin at a sensitivity of 0.5 g but switch (shown by broken line) to 1.0 and 2.0 g at the points marked a and b, respectively.

**Figure 3.** Dose dependency of norepinephrine-induced, endothelium-dependent relaxations in control and congestive heart failure vessels that displayed biphasic responses. The percent change in tension evoked by various concentrations of norepinephrine was calculated such that the reference point for 0% relaxation was the active force developed by a vessel immediately before the administration of a dose that elicited a relaxation. Return to baseline tension represents 100% relaxation. Open and solid bars depict vessels from control and heart failure dogs, respectively. \(*p<0.05, \text{heart failure vs. control. Data are from three control and eight heart failure dogs (n=17–36).} \)
In coronary arteries with an intact endothelium, acetylcholine produced dose-dependent relaxations in vessels precontracted with both KCl (20 mM) and PGF$_{2\alpha}$ (2 μM) (Figure 5). The magnitude of relaxation was independent of the contractile agent used, and no significant difference was observed between CHF and control segments. Acetylcholine relaxations were maximal at a concentration of 10 μM; further increments in dose either had no effect or caused contractions. Relaxations were never detected in denuded coronary vessels.

The capacity of the coronary vessels to relax to an endothelium-independent agent, as assessed by nitroglycerin responses, was comparable in control and CHF segments. Relaxation of 100% was achieved in all rings studied. Removal of the endothelium enhanced the potency of nitroglycerin to a similar extent in control and CHF rings, as reflected by a significant decrease in EC$_{50}$ values: control, 7.3 (5.5–9.7) versus 4.0 (3.3–4.8) nM, p<0.05, intact versus denuded, respectively; and CHF, 8.9 (7.3–10.8) versus 4.2 (3.4–5.1) nM, p<0.05, intact versus denuded, respectively.

**Discussion**

The present findings demonstrate that pacing-induced heart failure augments α-adrenergic-induced, endothelium-dependent relaxations in canine coronary arteries. The integrated response between α$_1$-adrenoeceptor–mediated contractions and α$_2$-adrenoeceptor–mediated relaxations is modified at peak heart failure, such that the constrictor signal is attenuated, whereas the dilator signal is potentiated.

This conclusion is based on the following observations: 1) denudation augmented norepinephrine-evoked contractions to a greater extent in CHF vessels relative to control vessels; 2) intact but not denuded coronary arteries responded to norepinephrine with slow, sustained contractions preceded by
rapid, reversible relaxations; 3) the relaxation component of the biphasic response occurred more frequently, required a lower threshold concentration of norepinephrine, and was of a greater magnitude in CHF arteries compared with control; 4) the maximal tension elicited by norepinephrine in coronary arteries was significantly reduced at peak CHF, but more so in intact as compared with denuded vessels; 5) contractions in response to the selective $\alpha_2$-adrenoceptor agonist methoxamine were diminished to a similar extent in both intact and denuded CHF preparations relative to control preparations; and 6) relaxations induced by BHT 920 in intact vessels were enhanced at peak CHF, whereas contractions generated in denuded preparations to the selective $\alpha_2$-agonist were depressed.

Previous investigators have demonstrated a loss of endothelium-dependent relaxations in hypertension and atherosclerosis. A decrease in the capacity of aortic and pulmonic vessels to relax in response to acetylcholine also has been noted in a rat model of CHF. Our investigation is unique in that it is the first report of an enhanced endothelial modulatory effect in a disease state.

Cocks and Angus found that in canine coronary arteries in vitro, norepinephrine-induced contractions were more powerful and sensitive in vessels whose endothelium had been mechanically removed. Although they concluded that norepinephrine could stimulate the release of EDRF, under normal resting tone, the contractile effect of norepinephrine consistently overrode the relaxation response to EDRF. In our study, the predominant response of intact coronary rings to $\alpha$-adrenergic stimulation likewise was vascular smooth muscle constriction. The stimulus for relaxation, however, was enhanced at peak CHF, such that norepinephrine-mediated relaxations became unmasked, directly opposing the contractile effect of the mixed $\alpha$-agonist. Biphasic norepinephrine responses were absent in denuded segments, suggesting that the initial relaxation component found in intact coronary arteries was mediated through an endothelium-dependent mechanism. The reversibility of the norepinephrine-induced relaxations is comparable to that observed for acetylcholine, further corroborating a role for EDRF. In addition to endothelium-dependent relaxations, slow sustained relaxations also were observed in both denuded as well as intact control and CHF rings at very high concentrations of norepinephrine (>10 $\mu$M). These relaxations, even in the presence of propranolol, were conceivably mediated by $\beta$-adrenoceptors because of the predominance of $\beta$-adrenoceptors in coronary arteries.

In the present study, the selective $\alpha_2$-adrenoceptor agonist BHT 920 produced relaxations in intact, but not denuded, control and CHF coronary arteries. Moreover, the $\alpha_2$-adrenoceptor agonist methoxamine failed to elicit biphasic responses in intact control or CHF rings, and the potency and efficacy of methoxamine was unaltered by removal of the endothelium. Such findings are compatible with the hypothesis that norepinephrine liberates the release of EDRF through stimulation of $\alpha_2$-adrenoceptors present on the endothelium.

The greater frequency and magnitude of norepinephrine-mediated, endothelium-dependent relaxations in vessels from dogs with CHF implies that in CHF, endothelial $\alpha_2$-adrenoceptor responsiveness is augmented. Enhancement of the $\alpha_2$-adrenoceptor-mediated relaxations probably accounts for the greater depression in the contractile response to norepinephrine in intact vessels relative to denuded vessels at peak CHF. It is unlikely that the observed effect is due to a general increase in the synthesis or release of EDRF at peak CHF, as relaxations to acetylcholine were similar in intact control and CHF vessels. However, the possibility that acetylcholine and norepinephrine stimulate the release of different relaxing factors cannot be excluded. Because relaxations induced by both acetylcholine and nitroglycerin were of a similar potency and magnitude in vessels from dogs with or without CHF, the present study argues against a heightened responsiveness of the underlying smooth muscle to one or more EDRFs.

The decrease in maximal tension produced by methoxamine and in preliminary studies by phenylephrine was not accompanied by a change in vessel sensitivity to the $\alpha_1$-agonists in CHF preparations. It has been proposed that under normal levels of sympathetic nerve input, vascular smooth muscle $\alpha_1$-adrenoceptor affinity is maintained at its lowest level, whereas receptor density is upregulated such that an increase in agonist concentration results in a decrease in receptor density without a change in receptor affinity. Thus, the elevated systemic levels of norepinephrine found in pacing-induced heart failure may result in a specific downregulation of coronary arterial $\alpha_1$-adrenoceptors, accounting for the observed desensitization to $\alpha_1$-adrenoceptor agonists in vitro. The concentration of catecholamines that stimulate vascular smooth muscle $\alpha_1$-adrenoceptors (exposed primarily to neuronal norepinephrine) and endothelial $\alpha_2$-adrenoceptors (exposed primarily to plasma norepinephrine) no doubt differs in CHF, and this may account for the dissimilar changes noted in the contractile and relaxation responses. Variations in the receptor/effector coupling mechanisms for coronary smooth muscle $\alpha_1$-adrenoceptors and endothelial $\alpha_2$-adrenoceptors also may play a role. Furthermore, we currently are unable to confirm whether changes in receptor density, affinity, or $G$ proteins are responsible for the observations outlined in this study.

Previous investigators have reported that canine epicardial coronary arteries contain a single population of $\alpha_1$-adrenoceptors. Hence, although the decrease in contractions evoked by BHT 920 in denuded segments at peak CHF indicates a possible downregulation of vascular smooth muscle $\alpha_2$-adrenoceptors, activation of $\alpha_2$-adrenoceptors by BHT 920, especially at high concentrations, cannot
be excluded. A general defect in the calcium mobilizing processes of coronary arteries at peak CHF may likewise be involved, because there was a trend toward a lower contractile response to KCl in both intact and denuded coronary arteries at peak CHF. In summary, pacing-induced CHF in the dog is associated with a shift in coronary $\alpha$-adrenergic constrictor/dilator balance, such that $\alpha_1$-adrenoceptor-mediated contractions are attenuated, and endothelium-dependent $\alpha_2$-adrenoceptor-mediated relaxations are enhanced. As coronary $\alpha$-adrenergic–induced constriction is capable of competing with metabolically induced vasodilatation, such an alteration may be an important protective response of the coronary vasculature in the setting of CHF. It is prudent to emphasize that the data were derived from isolated coronary arteries from male mongrel dogs with pacing-induced CHF; the implications for the intact human coronary circulation are unknown. Although the ultimate significance of these findings remains to be determined, it is clear that pathophysiological conditions, such as pacing-induced CHF, can lead to an increase in endothelial modulatory function.

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References

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