Norepinephrine Elicits β₂-Receptor–Mediated Dilation of Isolated Human Coronary Arterioles

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Background—The exact role of adrenoceptors in norepinephrine (NE)-mediated regulation of the human coronary circulation has yet to be elucidated. Thus, the goals of this study were to characterize the adrenoceptors involved in the responses to NE in isolated human coronary arterioles and small arteries.

Methods and Results—Arterioles (n=39) and small arteries from the left ventricle of explanted human hearts were isolated and cannulated. Vessels from the hearts of 21 patients were studied: 15 males and 6 females, aged 0.5 to 63 years. Nineteen patients were considered to be New York Heart Association class 4. All hearts exhibited hypertrophy (190±20%). The passive diameter of arterioles was 167±8 μm (range 97 to 323 μm). NE (10⁻⁷ to 3×10⁻⁷ mol/L) elicited concentration-dependent dilations (47±4 μm) that were unaffected by endothelium removal, Nω-nitro-L-arginine (10⁻⁴ mol/L, an NO synthase inhibitor), or practolol (10⁻⁶ mol/L, a β₁-receptor blocker). However, administration of propranolol (10⁻⁵ mol/L, a combined β₁- and β₂-receptor blocker) or butoxamine (10⁻⁶ mol/L, a β₂-receptor blocker) completely eliminated the NE-induced dilation. Constrictions to NE (2 of 39 vessels) were inhibited by prazosin (10⁻⁶ mol/L, an α₁-receptor blocker). Methoxamine (10⁻⁵ to 10⁻⁴ mol/L, an α₁-agonist) had no effect, whereas U44619, a thromboxane mimetic, elicited dose-dependent constriction of vessels.

Conclusions—Our data indicate that isolated human coronary arterioles and small arteries dilate to NE via β₂-receptors on smooth muscle. These findings are important to our understanding of the mechanisms action of NE in the human coronary circulation. (Circulation. 2002;106:550-555.)

Key Words: norepinephrine ■ receptors, adrenergic ■ arteries

Coronary arteriolar dysfunction can lead to cardiac ischemia and heart failure, frequently requiring cardiac transplantation. On the basis of primarily animal experiments, it has been learned that several mechanisms ensure the adequate blood supply of cardiac muscle by the fine regulation of coronary vascular tone.1 Obviously, less data are available from human studies. Recently, we and others2–3,5 explored the role of endothelial dilator factors, such as NO, in the regulation of coronary arterioles, including those isolated from explanted human hearts. However, it is known that in the absence of endothelial factors, the tone of vascular smooth muscle (and, hence, the diameter of arterioles) is still controlled by other local and remote mechanisms.

The role of norepinephrine (NE) in the genesis of coronary vasospasm and severe microvascular constriction, leading to ischemia in the coronary circulation, has been controversial. Original studies by Zuberbuhler and Bohr6 indicated that α-adrenergic receptor activation caused constriction of large coronary arteries. These and other systematic studies led to the concept that the coronary circulation constricts in the presence of NE and that this constriction may have both physiological and pathophysiological roles. For instance, Feigl7 and Murray and Vatner8 showed in the canine heart that α-receptor blockade can enhance the increase in coronary dilation during exercise. These results were attributed to α-adrenergic vasoconstriction limiting the increase in blood flow, which was thought to be primarily of metabolic origin. More recent studies, primarily from Chilian, Jones, and colleagues,9–12 defined the location of the constriction to NE in the canine heart to coronary microvessels >100 μm in diameter. In contrast, other studies indicated that β-adrenergic stimulation of the coronary circulation resulted in vasodilation and increases in coronary blood flow.13 Such findings led to the current hypothesis proposed by Miyashiro and Feigl14 that β-adrenergic receptors on blood vessels contribute to a “feedforward” coupling of metabolism and adrenergic responses in the physiological control of the coronary circulation. These disparate actions of NE in the
coronary circulation have led to a debate regarding the real physiological significance of NE in the control of vascular resistance in experimental animals and, by extrapolation, in the human heart.15

Importantly, NE can affect, in addition to the vascular wall, the functions of cardiac myocytes. Recent studies by Tiefenbacher et al16 have shown that responses of isolated canine coronary arterioles to NE are greatly modified by the presence or absence of cardiac muscle. The vascular response of arterioles to NE is further complicated by the fact that NE can affect α- and β-adrenoceptors located on the endothelium,17–25 smooth muscle,26–28 adventitia,29 and cardiac muscle.30 Aside from its direct effects in vivo, an indirect action of NE has also been documented; i.e., NE-induced constriction can increase wall shear stress, which, via the activation of endothelial factors, leads to dilation, modulating the original effect of NE.30

Collectively, these studies revealed that in vivo there are several mechanisms that can mask the direct vasomotor effects of NE on vascular diameter. Thus, it seemed important to characterize the complex actions of NE by elucidating its direct effects on the human coronary vascular wall in the absence of possibly confounding factors in vivo.31,32 Under normal conditions, the primary determinants of resistance in the coronary circulation are the small arteries and arterioles. Therefore, in the present study, we characterized responses to NE and the adrenergic receptors involved in the mechanism of action of NE in isolated human coronary microvessels.

Methods

Procurement of Human Hearts

Explanted hearts were harvested from patients (n = 21) at the time of orthotopic cardiac transplantation and immediately placed in iced normal saline in the operating room. The apical portion of each heart was removed for study; this included the left ventricular free wall, the septum, and a portion of the right ventricular free wall. In addition, hemodynamic information was collected on all patients. Nineteen patients had severe end-stage cardiac failure at the time of explantation; this included the left ventricular free wall, the septum, and a portion of the right ventricular free wall. In addition, hemodynamic information was collected on all patients. Nineteen patients had severe end-stage cardiac failure at the time of cardiac transplantation. Two patients had intact ventricular function and underwent transplantation for reasons other than ventricular failure.

Isolation of Arterioles

Experiments were conducted on arterioles and small arteries isolated from the left ventricle within a period of 2 to 3 hours after removal of the heart. A piece of cardiac tissue was excised and placed in a dissecting dish containing cold (0°C) physiological salt solution at pH 7.4. The salt solution contained (in mmol/L) NaCl 145, KCl 5.0, CaCl2 2.0, MgSO4 1.0, NaH2PO4 1.0, dextrose 5.0, pyruvate 2.0, EDTA 0.02, and MOPS 3.0. With the use of microscissors and an operating microscope (Olympus), segments of the intramyocardial arterioles were isolated from the cardiac tissue and transferred to the vessel chamber.1 Isolated arterioles were cannulated on 2 glass pipettes and suffused with physiological salt solution containing (in mmol/L) NaCl 118.0, KCl 5.0, CaCl2 2.5, MgSO4 1.0, KH2PO4 1.0, dextrose 10.0, NaHCO3 24.0, and EDTA 0.02, with 5% CO2 balanced with room air, at pH 7.4, in a vessel chamber. The vessels were warmed slowly to 37°C (YSI temperature controller). Intraluminal pressure was maintained constant at 60 mm Hg with a pressure-serve controller (Living System). The volume of the chamber and reservoir was 100 mL, and the rate of flow of the suffusion solution was 40 mL/min.

Internal diameter of vessels was measured with an image-shearing monitor (model 908, IPM) and recorded on a chart recorder (model MC6625, Multicorder). After 1 hour of equilibration, changes in diameter of arterioles and arteries in response to the abluminal administration of various agents were studied. All studies were conducted at 60 mm Hg intraluminal pressure with no intraluminal flow. The endothelium was removed by injection of air into the lumen of the arteriole.1 Removal of the endothelial cell layer was confirmed by the absence of a response to the endothelium-dependent dilator agent arachidonic acid (10−5 mol/L). A total of 39 vessels were studied, of which 16 (41%) developed spontaneous tone; those that did not were administered endothelin (~10−10 mol/L) to constrict the vessels to ~50% of their passive diameter.

Experimental Protocols

Responses to NE (10−7 to 3×10−7 mol/L) were assessed in each vessel before and after removal of endothelium or administration of 1 of the following inhibitors: N-nitro-l-arginine (L-NNA, 10−4 mol/L), an NO synthase inhibitor; propranolol (10−5 mol/L), a combined β1- and β2-adrenoceptor antagonist; butoxamine (10−6 mol/L), a β2-adrenoceptor antagonist; practolol (10−6 mol/L), a β1-adrenoceptor antagonist; and prazosin (10−6 mol/L), an α1-adrenoceptor antagonist. None of these drugs affected the basal diameter of these vessels significantly. Responses to salbutamol (10−6 mol/L), a β2-agonist, were also determined. To further characterize the contractile function of human coronary vessels, we tested the effects of the α1-agonists phenylephrine and methoxamine (10−9 to 10−3 mol/L) and the thromboxane mimetic U46619 (10−5 to 5×10−4 mol/L), known to elicit endothelium-independent constriction on vessels ranging in size from 150 to 650 μm in diameter.

At the conclusion of each experiment, the suffusion solution was changed to a Ca2+-free solution containing 1 mMol/L EGTA. Vessels were incubated for 10 minutes to obtain passive diameter at 60 mm Hg perfusion pressure. The passive diameter was used to assess the active tone generated by the arterioles.

Chemicals

All chemicals were obtained from Sigma Chemical Co and were dissolved in distilled water, except for U46619, which was dissolved in ethanol as a 10−2 mol/L stock solution. All solutions and drugs were prepared on the day of the experiments and further diluted with the suffusion solution.

Statistical Analysis

Data are presented as mean±SEM, with n indicating the number of vessels. Both absolute and normalized data were evaluated. Statistical significance was calculated by repeated-measures ANOVA. The Student t test was also used, as appropriate. The significance level was taken at P<0.05.

Results

Patient Population

Pertinent clinical data are listed in the Table. Of the 21 patients from whom hearts were harvested, 15 were male and 6 were female. The age range was 0.5 to 63 years, with a median age of 20.5 years. The most common clinical diagnosis was idiopathic dilated cardiomyopathy (12 patients). Nineteen hearts were designated as failing; these were obtained from patients whose main indication for transplantation was low cardiac output and failure of the left ventricle. Two hearts were nonfailing; one of these was obtained from a patient who was transplanted for cyanotic congenital heart disease not amenable to conventional surgery, and the other was from a patient who underwent retransplantation because of accelerated coronary vasculopathy of his first cardiac allograft. All patients were considered New York Heart Association class 4. Patients were treated with a combination of drugs, including dobutamine, digoxin, ACE inhibitors,
dopamine, antiarrhythmics, and heparin, until the time of transplantation.

On pathological examination, all hearts exhibited some degree of myocardial hypertrophy. This was quantified as the percentage above estimated normal heart weight for age and sex, which was more useful than absolute weight in grams because the population from which the explanted hearts were obtained included both pediatric and adult patients of both sexes. The mean percentage above normal heart weight was $190\pm20\%$.

Responses of Arterioles to NE
The average passive diameter of arterioles in the presence of 60 mm Hg intraluminal pressure was $167\mu m$ (range 97 to 323 $\mu m$, n=39). Sixteen vessels developed spontaneous tone. The active and passive diameters and the tone of these vessels were $72\mu m$ (range 43 to 209 $\mu m$), $156\mu m$ (range 127 to 323 $\mu m$), and $45\mu m$ (range 26.2% to 2.6%, respectively. NE (10$^{-7}$ to 3 $\times$ 10$^{-7}$ mol/L) elicited dose-dependent dilations of arterioles (n=39) (Figure 1, top). The responses of individual arterioles to all 3 concentrations of NE are plotted in Figure 1 (bottom). The data show that the percent increase in vessel diameter was not dependent on vessel size. There were no significant differences in NE-induced dilations between vessels with spontaneous tone and vessels treated with endothelin. Only 2 vessels (average passive diameter 240 $\mu m$) responded with a decrease in diameter to NE (see below).

Role of Endothelium
Removal of endothelium (Figure 2, top) or administration of L-NNA (10$^{-4}$ mol/L) (Figure 2, bottom) did not affect the dilator responses of human coronary vessels to NE.

Role of $\beta$-Receptors
The administration of propranolol (10$^{-4}$ mol/L), but not practolol (10$^{-6}$ mol/L), completely eliminated the dilator responses induced by NE (Figure 3, top and middle, respectively). To further characterize the role of $\beta$-receptors, butoxamine (10$^{-6}$ mol/L) was administered. The data clearly show that butoxamine completely eliminated the NE-induced

![Figure 1](http://circ.ahajournals.org/)

![Figure 2](http://circ.ahajournals.org/)

![Figure 3](http://circ.ahajournals.org/)
dilations (Figure 3, bottom). In addition, we have found that salbutamol \(10^{-6}\) mol/L elicited substantial dilation of human coronary arterioles (60\% decrease in diameter).

**Role of \(\alpha\)-Receptors**

Phenylephrine \((n=5)\) did not cause a significant change in the diameter of either endothelium-intact or endothelium-removed coronary vessels. To further characterize the presence of \(\alpha\)-adrenoceptors on human coronary arteries, we administered methoxamine. Interestingly, methoxamine elicited constriction in only 2 arteries and only at higher concentrations (Figure 4, top). To test the ability of isolated coronary arteries to constrict, we also investigated the effects of U46619, which elicited concentration-dependent dilations, responses that are mediated by \(\beta_2\)-receptors on smooth muscle.

Figure 3. Changes in diameter of isolated human coronary arterioles in response to NE in presence of propranolol (top), prazosin (middle), and butoxamine (bottom). Data are mean±SEM.

Figure 4. Changes in diameter of individual isolated human coronary arteries in response to methoxamine (top) and thromboxane mimetic U46619 (bottom). Insert, Percent constriction to U46619. Data are mean±SEM \((n=8 [\text{methoxamine}] \text{ and } 6 \text{[U46619]})\).

mol/L NE), we found that prazosin \((10^{-6} \text{ mol/L})\) eliminated these responses (constrictor responses were converted to 1- and 3-\(\mu\)m increases in diameter). It should be noted that these 2 (of a total of 39) ventricular small arteries that exhibited a modest constriction to NE were both obtained from the heart of a patient with dilated cardiomyopathy, from which an additional vessel dilated to NE.

**Discussion**

The new finding of the present study is that in isolated coronary arterioles and small arteries obtained from the left ventricle of explanted human hearts, NE elicits concentration-dependent dilations, responses that are mediated by \(\beta_2\)-receptors on smooth muscle.

In the coronary circulation, activation of adrenoceptors plays an important role in the regulation of vascular resistance.\(^6\text{–}^{13}\) Previous studies established the presence of \(\alpha_1\text{-, }\alpha_2\text{-, }\beta_1\text{-, and }\beta_2\text{-adrenoceptors in coronary vessels.}\(^6\text{–}^{17}\) One of the important endogenous substances that binds to these receptors is NE, released from nerve terminals adjacent to the vessels.\(^{17,29}\) Previous studies have already documented that \(\beta\)-adrenergic blockade can potentiate coronary artery constriction in patients with coronary artery disease, which is most likely mediated by unopposed \(\alpha\)-adrenergic vasomotor tone.\(^{33}\) In addition, it has been shown that adrenergically mediated coronary vascular tone may contribute to ischemia in patients with coronary disease.\(^{34}\)

Responses of coronary arterioles and small arteries to NE have been investigated by many studies in vivo and in vitro, revealing that adrenoceptors are located on several cell types and tissues, including endothelium,\(^4\text{,}^{17\text{–}28}\) smooth muscle,\(^{26\text{–}28}\)
adventitia, 29 and cardiac muscle, 14 and that all can be stimulated by NE. Evidence shows that activation of \( \alpha_1 \)- and \( \alpha_2 \)-receptors in vivo results in constriction of the coronary vessels, 15,17,26 whereas activation of \( \beta_1 \)- and \( \beta_2 \)-receptors elicits dilations. 17,21–25 In vivo investigation of the mechanism of action of NE infused in the coronary circulation has been hampered somewhat by the actions of NE on various adrenoceptors and tissues. For example, NE can stimulate \( \beta_1 \)-receptors on cardiac muscle, eliciting an increase in metabolism that leads to metabolic dilation. 12,17 In addition, the action of NE on vascular \( \alpha_1 \)- and \( \alpha_2 \)-receptors elicits constriction, thus opposing metabolic vasodilation. 9,17 Interestingly, this latter effect has been difficult to demonstrate in vitro experiments, although Toda 27 has shown that NE causes constriction of strips of human epicardial coronary arteries. In contrast, Jones et al. 11 reported that NE elicited either no response or only a slight dilation of isolated canine coronary arterioles. Because of the lack of constriction to this agent, these authors hypothesized that during isolation, the vessels may have lost their \( \alpha \)-receptors. It has also been shown that the number of \( \alpha_1 \)-receptors increases with the size of the vessels, whereas the number of \( \alpha_2 \)-receptors decreases. 9 In this context, Jones et al. have also demonstrated that in dogs, in vivo, NE infusion elicits constriction in vessels >100 \( \mu \)m in diameter and dilation in vessels <100 \( \mu \)m in diameter. Because small arteries and arterioles determine the resistance of the coronary circulation, it seemed important to elucidate the direct action of NE and the receptors involved in the response to NE in these vessels. Also, because most of the above-mentioned data were obtained in animal experiments, revealing a species heterogeneity in response to NE, in the present study, we aimed to examine the direct vascular action of this agent in isolated microvessels of explanted human hearts.

The substantial constrictor responses of coronary arteries to the thromboxane mimetic U46619 indicate that these vessels are capable of constriction in spite of their lack of constrictor responsiveness to the specific \( \alpha_1 \)-receptor agonists phenylephrine and methoxamine (Figure 4). These data do not necessarily imply that human small coronary arteries do not have or that they have lost their \( \alpha \)-receptors in vitro, but the data do indicate that the vessels remain viable and that the ability of smooth muscle to constrict is maintained during the isolation procedure. The lack of a response to stimulation of \( \alpha \)-receptors in vitro is not completely surprising, inasmuch as previous studies in canine coronary vessels by Jones et al. 11 found similar results.

The salient finding in the present study is that human coronary arterioles and small arteries respond with dilation to NE. This is in contrast to many in vivo studies in which constriction to NE was observed. 9 A recent study in canine coronary arterioles by Tiefenbacher et al. 10 offered a possible explanation for these controversial observations. This group demonstrated that in the presence of cardiac myocytes, arterioles constrict, whereas in their absence, they fail to constrict in response to phenylephrine. Elucidating further the mechanism of action of phenylephrine, they showed that by acting on cardiac muscle, NE elicits the release of an unidentified factor that stimulates the release of endothelin from endothelial cells. This seemingly complex pathway may be responsible for \( \alpha \)-adrenergic coronary constriction in vivo. As of today, however, there have not been any studies of human coronary arterioles in vitro to assess the direct vascular actions of NE.

We found that concentration-dependent increases in the diameter of arterioles in response to NE were not affected by the removal of the endothelium or administration of L-NNA. This finding is somewhat in contrast to previous reports suggesting that NE, by acting on endothelial \( \alpha_1 \)-receptors, elicits the release of NO, which would counteract the direct constrictor effect of NE. 18,32 Indeed, our previous studies showed that the endothelium of canine coronary arterioles is able to synthesize NO, a capacity that may be reduced in the presence of heart failure. 1,32 The lack of an effect of removal of endothelium and L-NNA administration on basal arteriolar diameter and responses to NE in the present study reflects, most likely, the low level of NO released from a single coronary arteriole under no-flow conditions and, possibly, the presence of heart failure in the patients.

As for the receptor(s) involved in the dilator mechanism activated by NE, we found that propranolol, a combined \( \beta_1 \)- and \( \beta_2 \)-receptor blocker, inhibited NE-induced dilator responses. Because butoxamine, a specific \( \beta_2 \)-receptor blocker, but not practolol, a specific \( \beta_1 \)-receptor blocker, eliminated completely the dilator responses induced by NE, we concluded that NE elicited the dilation of human coronary arterioles via \( \beta_2 \)-receptors on arteriolar smooth muscle. The presence and importance of \( \beta \)-receptors in human coronary arterioles are further underscored by the finding that salbutamol, a \( \beta_2 \)-receptor agonist, also elicited dilation. The present results are also in accord with the recent reports of Gorman and colleagues 35,36 who demonstrated in exercising dogs a significant role for feedforward \( \beta \)-receptor–mediated sympathetic coronary vasodilation.

Obviously, the present experiments do not have a control in a sense that can be expected in animal experiments. Thus, we do not know with any certainty what the response to NE of isolated coronary arterioles in healthy young individuals would be, inasmuch as these vessels were isolated from diseased hearts. But regardless of the etiology of cardiac failure or the pretransplantation drug regimen that the patients received, they responded to the administration of NE with vigorous dilation. The patient population included 19 patients with symptomatic severe congestive heart failure and 2 patients with nonfailing hearts (Table). The coronary arteries of these 2 patients with qualitatively normal ventricular function also dilated in response to NE. Thus, ventricular arterioles and small arteries seem to have responded to NE in essentially a uniform fashion. Although the reasons for and the significance of the pure \( \beta_2 \)-receptor–mediated coronary dilation is not entirely clear, it may be related to an alteration in vascular adrenergic receptors secondary to the development of heart failure, or, more likely, it may reflect the normal behavior of coronary arterioles in which, unlike in larger vessels, \( \beta_2 \)-receptors predominate to mediate the dilation.

In conclusion, the present study demonstrates that in human coronary arterioles and small arteries isolated from the
left ventricle, NE elicits dilation via stimulation of β2-receptors on smooth muscle. Although these studies were conducted on vessels obtained from failing hearts, these collective findings advance our understanding of the regulation of human coronary blood flow and, thus, may aid in therapy for cardiovascular disorders.

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