Modulation of Endothelin-1 Coronary Vasoconstriction in Spontaneously Hypertensive Rats by the Nitric Oxide System

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To determine whether nitric oxide contributes to the augmented vasoconstrictive response to endothelin-1 (ET-1) in coronary vessels of hypertensive hearts, and also whether L-arginine administration can inhibit the augmented response to ET-1, we designed experiments to measure coronary perfusion resistance in isolated hearts of spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY) with or without L-arginine administration (0.5 g/L) for 2 weeks. The hearts were paced at a constant rate and perfused by the Langendorff technique at constant pressure (75 mm Hg). Perfusion flow and pressure were monitored, and coronary vascular resistance (CVR) was calculated. ET-1 infusion elicited dose-dependent increases in CVR in both WKY and SHR. At an ET-1 concentration of $1.5 \times 10^{-9}$ mol/L, the response was significantly greater in SHR. In L-NAME–treated WKY and SHR, responses to ET-1 were augmented, compared with those of nontreated rats, and this augmentation was greater in WKY. L-arginine administration reduced the CVR response to ET-1 in SHR, whereas it did not change responses to ET-1 in WKY. These findings suggest that the augmented vasoconstriction of the coronary artery induced by ET-1 in hypertensive hearts was due to a reduction in nitric oxide release in coronary vessels and that L-arginine can partially inhibit the vasoconstrictive response of the coronary artery. Am J Hypertens 2000;13:83–87 © 2000 American Journal of Hypertension, Ltd.

KEY WORDS: Endothelin-1, coronary artery, spontaneously hypertensive rats, L-arginine, L-NAME, hypertensive heart.

Essential hypertension is often associated with impairment of the coronary circulation. This impairment is partly due to functional abnormalities such as defective endothelium-mediated control of vascular tone. The vascular endothelium regulates regional vascular tone by release of vasodilator substances such as endothelium-derived relaxing factor (EDRF) and vasoconstrictor substances such as endothelin. Endothelin-1 (ET-1) exerts potent vasoconstriction activity in experimental animals and in humans, and endothelin produces coronary vasoconstriction at pharmacologic and

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pathophysiologic concentrations. Recently, we demonstrated that the vasoconstrictive response to ET-1 in coronary vessels was increased in hypertensive rats compared with normotensive rats. Nitric oxide (NO) contributes to maintain coronary artery tone. An interaction between endothelin and NO may occur in the coronary vessels. Indeed, an NO inhibitor has been shown to result in augmented vasoconstriction by ET-1 in the coronary artery of the goat and rat. Thus, NO apparently counteracts the vasoconstriction produced by ET-1 in a coronary artery. The administration of L-arginine, which is a precursor of NO, reverses the elevation of blood pressure caused by chronic administration of N^G-monomethyl-L-arginine monoacetate (L-NMMA). L-arginine normalizes endothelial function in cerebral vessels from hypercholesterolemic rabbits. In the coronary artery, it improves the endothelium-dependent vasodilatation in hypercholesterolemic humans and in patients with angina pectoris. Thus, L-arginine can improve the endothelium-dependent vasodilatation of the coronary artery. If the augmented vasoconstriction by ET-1 is from decreased NO release in the coronary artery, it may be possible to reduce this augmentation by administration of L-arginine.

The present study was designed to confirm the augmented vasoconstrictive response to ET-1 in hypertension, to determine whether NO can counteract this vasoconstrictive response, and to discover whether chronic L-arginine administration can improve the altered responses to ET-1 in the coronary artery in hypertension.

MATERIALS AND METHODS

Nine- to 12-week-old spontaneously hypertensive rats (SHR) and Wistar-Kyoto rats (WKY) weighing 230 to 280 g were used as the experimental subjects. The WKY and SHR were divided into three subgroups; one group received no treatment, one was treated with L-N^G-nitro arginine methyl ester (L-NAME), and the third was given L-arginine. L-NAME (75 mg/dL) and L-arginine (0.5 g/L) were added to tap water. After 2 weeks of treatment, vasoconstrictive responses to ET-1 in isolated rat hearts were compared between the groups. Systolic blood pressure was measured by the tail-cuff method once a week. Each heart was excised under anesthesia with sodium pentobarbital (50 mg/kg body weight, intraperitoneal), and the isolated heart was perfused at a constant pressure (75 mm Hg) with a Langendorff apparatus. The perfusion solution was Krebs-Henseleit solution at 37°C oxygenated by 95% O2 and 5% CO2, with the addition of (mmol/L) 118 NaCl, 4.7 KCl, 2.5 CaCl2, 1.2 MgSO4, 1.2 KH2PO4, 25 NaHCO3, 0.5 Na2EDTA, and 11.1 glucose. The heart rate was paced at 300 beats/min using an electric stimulator. Coronary perfusion pressure was recorded with a pressure transducer, and coronary perfusion flow was measured using a digital drop counter. Coronary vascular resistance (CVR) was calculated by coronary pressure and flow. L-NMMA (10 – 4 mol/L), and ET-1 (1.5 × 10 – 10, 1.5 × 10 – 9, 3 × 10 – 9 mol/L) were infused in the perfusate. The total heart and the left ventricle were weighed separately.

Data are presented as mean ± SEM. ANOVA followed by Fisher PLSD for repeated measurements was used for statistical analysis. A two-tailed value of P < .05 was considered to indicate statistical difference.

RESULTS

Augmented Vasoconstrictive Responses to ET-1 in Hypertensive Hearts Systolic blood pressure was significantly higher in SHR than in WKY (126 ± 6 vs 180 ± 8 mm Hg, P < .001). The weight of left ventricle per 100 g of body weight was significantly greater in SHR than in WKY (0.219 ± 0.003 vs 0.282 ± 0.005, P < .0001). The infusion of ET-1 dose-dependently increased CVR in both normotensive and hypertensive hearts; however, the responses were significantly greater in the hypertensive hearts (Figure 1).

L-NAME Treatment Enhances Vasoconstrictive Responses to ET-1 After administration of L-NAME for 2 weeks, systolic blood pressure was elevated in both WKY and SHR. (144 ± 32 vs 166 ± 3 mm Hg, P < .05, and 181 ± 3 vs 204 ± 7 mm Hg, P < .05, for WKY and SHR, respectively). CVR responses to ET-1 were significantly enhanced in both normotensive and hypertensive hearts with L-NAME treatment, compared with those without the treatment. his enhancement was greater in normotensive than in hypertensive hearts (Figure 2).

L-Arginine Treatment Partially Reverses Vasoconstrictive Responses to ET-1 L-arginine administration did not significantly affect the systolic blood pressure in hypertensive (192.5 ± 3.4 vs 191.4 ± 5.2 mm Hg;
NS) or normotensive rats (126 ± 6 vs 128 ± 4, NS). However, CVR response to ET-1 was reduced significantly in SHR after L-arginine treatment, whereas response was not altered in WKY (Figure 3). The differences between SHR and WKY in the constrictive responses to ET-1 disappeared after L-arginine administration.

**DISCUSSION**

The present results show that vasoconstrictive response to ET-1 was augmented in the coronary artery of hypertensive hearts and that the reduction in NO release in the coronary vessels might contribute to this augmentation. Although the responses to ET-1 were enhanced in normotensive and hypertensive rats by chronic L-NAME administration, this enhancement was significantly larger in normotensive rats. Therefore, it assumes that NO counteracts the vasoconstriction induced by ET-1 quite distinctly in WKY. The reduction of NO release in coronary vessels in SHR was recovered by L-arginine administration. Then, reduced NO release mediated the augmented vasoconstriction by ET-1 in hypertensive hearts. ET-1 has been shown to induce a vasoconstrictive response in coronary artery in pig, dog, rats, and humans. The vascular effect of ET-1 is mediated by endothelin receptors. ET_A and ET_B receptors may be located in smooth muscle cells and can mediate vascular contraction. ET-1 induces increases in coronary perfusion pressure, and these increases are inhibited by ET_A receptor antagonist in isolated rat heart and porcine heart. ET_B receptors also are involved in vasoconstriction by ET-1 in rats and dogs. In results from isolated coronary artery specimens, contractile responses to ET-1 are neither augmented nor decreased in the coronary artery of SHR. Our present results indicate that vasoconstrictive responses to ET-1 are augmented in isolated perfused heart of SHR. We have no definitive explanation for the discrepancy between the previous results and our results. One possible factor is differences in experimental methods. Fuch et al used isolated left ventricular coronary arteries (200 to 300 microns diameter) and directly evaluate intraluminal diameter and other study used aortic ring. The augmented response to ET-1 in SHR was mediated by both ET_A and ET_B receptors, as previously demonstrated.

L-NAME administration enhanced the vasoconstrictive responses to ET-1 in both WKY and SHR, which is in agreement with a previous report in which L-NAME administration increased the responses to ET-1 in the coronary artery in goats. These findings indicate that NO can counteract the ET-1-induced vasoconstriction. In our results, coronary NO release seemed to be diminished in SHR because the enhancement of vasoconstrictive responses to ET-1 was smaller in L-NAME-treated SHR than in L-NAME-treated WKY. In another recent report, Crabos et al showed that basal NO release was reduced in the

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**FIGURE 2.** L-NAME treatment enhanced vasoconstrictive responses to ET-1 in the hearts of WKY and SHR rats. This enhancement was greater in normotensive hearts than in hearts of SHR.

**FIGURE 3.** (A) L-Arginine treatment restored vasoconstrictive responses to ET-1 in SHR rat hearts. (B) After L-arginine administration, the differences between SHR and WKY in the constrictive responses to ET-1 disappeared.
coronary artery of SHR. Tschudi and Luscher showed a blunted NO release stimulated by acetylcholine in the coronary artery from SHR, whereas Kelm et al demonstrated that NO release increased in the coronary artery of SHR. The reason for this discrepancy is unclear. In a clinical trial, Egashira et al showed that increases in coronary blood flow with both acetylcholine and substance P were blunted significantly in hypertensive patients compared with control subjects. This finding suggests that diminished NO release exists in hypertensive patients and was supported recently by another study. Additionally, a high level of ET-1 was reported recently in hypertensive patients, especially in those with coronary disease. Therefore, vasoconstriction by ET-1 may contribute to the impairment of coronary circulation in hypertension.

The acute administration of L-arginine has been shown to improve forearm and cerebral blood flow in hypercholesterolemic subjects in response to the endothelium-dependent vasodilator acetylcholine. In addition, acute and chronic L-arginine administration improves vasomotor function in animal models of hypercholesterolemia, suggesting an absolute L-arginine deficiency or, alternatively, a relative substrate deficiency resulting in a functional decrease in endothelial nitric oxide synthase (eNOS) activity. In this study, L-arginine was administered for 2 weeks. eNOS activity may be increased.

In human subjects, endothelial dysfunction, in the absence of angiographically significant coronary disease, is characterized by increased levels of circulating ET and decreased coronary NO release. Therefore, the relative imbalance of their vasomotor pathways in hypertension may conceivably cause myocardial ischemia in the absence of significant epicardial coronary stenoses.

REFERENCES


