

# Role of Nitric Oxide in the Regulation of Coronary Vascular Tone in Hearts From Hypertensive Rats

## Maintenance of Nitric Oxide-Forming Capacity and Increased Basal Production of Nitric Oxide

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**Abstract** In arterial hypertension, coronary flow reserve, expressed by the difference between autoregulated and maximal coronary flow, is frequently impaired. Previous experimental and clinical investigations using acetylcholine as a stimulus for the production of endothelium-derived relaxing factor suggested that an impaired endothelium-dependent vasodilation, presumably caused by a decreased formation of nitric oxide (NO), may account for this microvascular dysfunction. However, so far no study has been performed that quantifies the formation of NO within the coronary circulation of hypertensive hearts to assess its role in setting coronary vascular tone in the hypertensive heart. We therefore quantified NO formation within the coronary circulation of constant flow-perfused, isolated hearts from spontaneously hypertensive rats (SHR, 16th to 26th week), as a model for hypertensive heart disease, and from the normotensive control strain (Wistar-Kyoto, WKY) using the oxyhemoglobin technique. Coronary perfusion pressure and vascular resistance were almost 30% higher in SHR compared with WKY hearts. Intracoronarily applied NO decreased coronary vascular resistance by maximally 45% of resting values in a concentration-dependent manner in both

groups. The bradykinin-induced decrease in coronary vascular resistance and the parallel increase in NO release were comparable in SHR and WKY hearts and fell within the vasodilator range of exogenously applied NO. Moreover, basal release of NO normalized to heart wet weight was 50% higher in SHR compared with WKY hearts. Rates of basal NO release were correlated inversely with changes in coronary perfusion pressure and vascular resistance in both groups ( $r = -.85$  and  $-.84$ , respectively,  $P < .05$ ). This relation between resting coronary vascular resistance and NO formation, as a critical determinant of coronary flow, was shifted significantly to higher levels in SHR. From the present data we conclude that in the coronary circulation of SHR, NO formation is preserved under basal and stimulated conditions and critically determines resting coronary resistance. Moreover, the enhanced basal release of NO may serve the purpose of compensating the higher coronary vascular resistance of hypertensive hearts. (*Hypertension*. 1995;25:186-193.)

**Key Words** • endothelium-derived relaxing factor • nitric oxide • hypertension, arterial • coronary circulation • bradykinin

The endothelium contributes to the control of vascular smooth muscle tone by production and release of nitric oxide (NO), which accounts for the biological activity of the endothelium-derived relaxing factor (EDRF) (for review, see References 1 through 3). NO is produced by oxidation of the terminal guanidino nitrogen of L-arginine by an enzyme, the NO synthase (for review, see Reference 3). The development of guanidino-modified arginine analogues as specific inhibitors of NO synthase allowed the study of the effects of basal NO release on the resting tone of resistance vessels and its involvement in blood pressure regulation.<sup>4</sup> Since the first demonstration of a remarkable increase in arterial blood pressure after systemic application of NO synthase inhibitors in the rabbit,<sup>4</sup> the possible involvement of NO in the develop-

ment or maintenance of arterial hypertension has been discussed.

The formation and release of endothelium-derived NO into the coronary circulation has been quantified in isolated perfused hearts from the guinea pig<sup>5</sup> and rabbit.<sup>6</sup> Basal release of NO may be increased severalfold during infusion of endothelium-dependent vasodilators such as bradykinin, acetylcholine, serotonin, and ATP or as a result of an elevated perfusion flow in response to enhanced shear stress.<sup>7</sup> In hypertensive patients with and without left ventricular hypertrophy, the coronary flow reserve, determined by endothelium-dependent and -independent vasodilators, is frequently impaired.<sup>8-10</sup> So far, no experimental or clinical study has been published that quantifies cardiac NO release directly. Thus, a possible pathophysiological role of NO in the regulation of coronary vessel tone in arterial hypertension remains unclear.

Coronary vascular resistance (CVR) of hearts from spontaneously hypertensive rats (SHR), a model for genetically determined hypertension, is significantly higher than that of hearts from the normotensive Wistar-Kyoto (WKY) control strain. Whether or not an altered NO metabolism may account for this difference is unknown. A smaller NO production could potentially

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explain the higher coronary resistance. Alternatively, at comparable production rates, a faster inactivation of NO, eg, by reaction with superoxide anions,<sup>11</sup> could lead to a higher coronary resistance and/or a lower sensitivity of vascular smooth muscles to NO.<sup>12</sup>

The aim of the present study was to investigate the role of NO in the control of CVR in hypertensive hearts. Using isolated perfused hearts from SHR and WKY rats, we determined (1) the effects of exogenously applied NO on CVR, (2) the release of endogenously produced NO under basal and stimulated conditions, and (3) the effects of NO synthase inhibitors on NO production.

## Methods

### Preparation of Isolated Hearts and Experimental Protocol

SHR and WKY rats (Charles River, Kisslegg, FRG) in the 16th to 22nd week served as an experimental model for genetically determined arterial hypertension (each  $n=17$ ). Hearts were rapidly excised from diethylether-anesthetized rats according to the guidelines of the local committee for the use of animals in research. Perfusion was performed in a retrograde manner via the aorta at a constant flow rate of 14 mL/min with a modified Krebs-Henseleit solution as described in detail elsewhere.<sup>7</sup> With the use of this flow rate, a stable preparation was achieved with hearts from either WKY rats or SHR without changes in the inotropic parameters, left ventricular pressure and  $dP/dt_{max}$  for up to 2 hours. Moreover, coronary vascular reactivity on bolus infusion of adenosine (20  $\mu$ L, 100  $\mu$ mol/L) was similar at the beginning and end of each experiment. Indomethacin (10  $\mu$ mol/L) was added to the perfusion medium to prevent production and release of prostacyclin. In experiments in which NO release was determined, the perfusion medium was supplemented with 5  $\mu$ mol/L oxyhemoglobin (see below). Left ventricular systolic pressure (LVP) and its first derivative,  $dP/dt_{max}$ , were continuously measured with a Millar tip catheter inserted into the left ventricle. Coronary perfusion pressure (CPP) was continuously measured in the perfusion cannula 2 cm above the orifice of the coronary vessels. CVR was calculated as the ratio of CPP and flow. The hemodynamic parameters were sampled on a PD-11 computer and documented on a Watanabe linear recorder. Hearts were used for further study only when initial CPP was higher than 70 mm Hg and the bolus injection of adenosine decreased basal CPP by more than 25%. Rejected hearts amounted to 25% in the SHR and 15% to 20% in the WKY groups. At the end of each experiment, hearts were removed from the perfusion cannula, and wet weight was determined after gentle blotting of the endocardial and epicardial surfaces with a paper towel. All compounds infused to modulate NO release were given through a stainless steel cannula placed into the aortic inflow line in the vicinity of the coronary orifices at a rate of 200  $\mu$ L/min or less. The experimental protocol was started when LVP,  $dP/dt$ , CPP, and heart rate had reached stable baseline values, usually 20 to 30 minutes after artificial perfusion was started.

A concentration-response curve for the dilator effect of intracoronarily infused NO was derived by intracoronary infusion of authentic NO standards. Standards of aqueous NO solution were prepared as previously described.<sup>7,13</sup> Briefly, NO was dissolved in argon-degassed purified water, and the concentration of this stock solution was routinely determined by high-performance liquid chromatography (HPLC) via its degradation product nitrite. NO stability in these solutions was regularly checked either using a chemiluminescence technique without reductive preprocessing (CLC 750 TR, ECO Systems) or using the difference-spectrophotometric assay in a stop-flow technique to determine NO content via the molar extinction coefficient for the NO-induced conversion of oxyhemoglobin to methemoglobin, as previously described.<sup>14</sup>

Thereafter, NO solutions were diluted to the desired concentration under argon and kept in gas-tight syringes. The stability of prepared NO solution was maintained over more than 6 hours. For application of NO, these syringes were mounted in a precision infusion pump (Infors AG) and connected to an HPLC steel cannula, which was placed in the aortic inflow line directly in front of the ostium of the coronary arteries to limit destruction of NO during its transport to the tissue. To stimulate endogenous NO production, we used bradykinin at intracoronary final concentrations of  $10^{-8}$  to  $10^{-7}$  mol/L. The stereospecific inhibitor *N*<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME) was infused at a final concentration of  $10^{-4}$  mol/L to block endothelial NO production. Bradykinin, indomethacin, adenosine, bovine hemoglobin, and L-NAME were obtained from Sigma Chemical Co.

### NO Measurement

The experimental setup and method used to quantify NO release into the coronary circulation of isolated hearts has been described in detail elsewhere.<sup>7,15</sup> Briefly, an aliquot (9 mL/min) of the coronary effluent perfusate dripping from the hearts of either WKY rats or SHR placed in a water-jacketed chamber was transferred by means of a roller pump to the flow-through cell of a double-beam dual-wavelength photometer (Shimadzu UV 3000). The spectrophotometric quantification of NO is based on the equimolar conversion of oxyhemoglobin to methemoglobin by NO.<sup>15</sup> Continuous measurement of NO release was achieved by the time-dependent recording of the absorption difference between the absorption maximum and the isosbestic point of the difference spectrum of oxyhemoglobin versus methemoglobin (401 versus 411 nm). Kinetics, sensitivity, specificity, and linearity of the assay for the quantification of NO release into the coronary circulation have been described extensively elsewhere.<sup>7</sup>

### Statistics

Basal CPP of isolated hearts from either WKY rats or SHR revealed an even distribution using a Gaussian function. Differences between either SHR and WKY hearts or control and the respective vasodilator response (changes in CPP and CVR) after application of authentic NO or bradykinin within each group were evaluated by one-way ANOVA for repeated measures. To test for significance of differences in basal NO formation and basal hemodynamic parameters between SHR and WKY hearts, we applied Student's two-tailed *t* test for unpaired data. Two-sided probability values of less than or equal to .05 were considered significant. The relationship between basal NO formation and basal coronary vascular tone was evaluated using a linear univariate regression analysis. Data are given for the regression (*b*), correlation (*r*), and determination (*r*<sup>2</sup>) coefficient (see Fig 7). Data processing was performed with the SPSS software package (release 5.0.1, SPSS, Inc). Values are given as the mean  $\pm$  SEM; numbers in parentheses indicate the number of hearts tested with the respective intervention.

## Results

Table 1 gives basal hemodynamic parameters of isolated WKY or SHR hearts perfused with a constant flow of 14 mL/min. Although heart rate was comparable in both groups, LVP, and thus  $dP/dt_{max}$ , was approximately 30% higher in SHR compared with WKY hearts. CPP was significantly higher in the SHR group, resulting in a 32% higher CVR in SHR compared with WKY hearts under resting conditions. The wet heart weight determined at the end of each experiment was significantly higher in SHR, corresponding to a 60% higher ratio of heart weight to body weight in SHR compared with WKY rats.

### Effect of NO Release on Coronary Hemodynamics

Intracoronarily infused NO dilated coronary resistance vessels in a concentration-dependent manner (see

TABLE 1. Basal Hemodynamic Parameters of Isolated Hearts

Parameter	WKY	SHR
CPP, mm Hg	100±5	137±6*
CVR, mm Hg · min/mL	7.4±0.3	9.8±0.5*
CVR <sub>g</sub> , mm Hg · min/mL · g	6.8±0.4	7.4±0.4*
LVP, mm Hg	94±2	128±5*
dP/dt <sub>max</sub> , mm Hg/s	5711±145	7411±213*
HR, bpm	243±4	251±6
Heart wet weight, g	1.04±0.02	1.3±0.03*
Body weight, g	381±22.5	305±4.5*
Heart-body weight ratio, %	2.7±0.09	4.3±0.7*

WKY indicates Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; CPP, coronary perfusion pressure; CVR, coronary vascular resistance; CVR<sub>g</sub>, CVR normalized to heart weight; LVP, left ventricular systolic pressure; and HR, heart rate. Isolated WKY and SHR hearts (each n=17) were perfused at constant flow (14 mL/min).

\*P<.05, WKY vs SHR.

Fig 1). NO is a powerful coronary vasodilator, as indicated by the low threshold concentration of 0.1 nmol/L. Already at a concentration of 0.3 nmol/L, CPP was significantly decreased compared with control values within each group. Half-maximal effects were achieved at approximately 1 nmol/L NO. Onset of coronary vasodilation was obtained always within less than 1.5 seconds after the start of NO infusion (corrected for transit time of dead space). In both groups, NO did not significantly affect LVP, dP/dt<sub>max</sub>, or heart rate at the concentrations used. Control values for CPP and CVR were significantly higher in SHR compared with WKY hearts (see Table 1). The absolute drop in CPP and CVR was consistently higher in SHR than in WKY hearts. However, when the concentration-response curves for NO-induced changes in CPP were normalized to the respective control value for CPP in each group, the curves were superimposable (see Fig 1), indicating that the sensitivity to NO of the coronary vascular smooth muscle was identical in both groups.

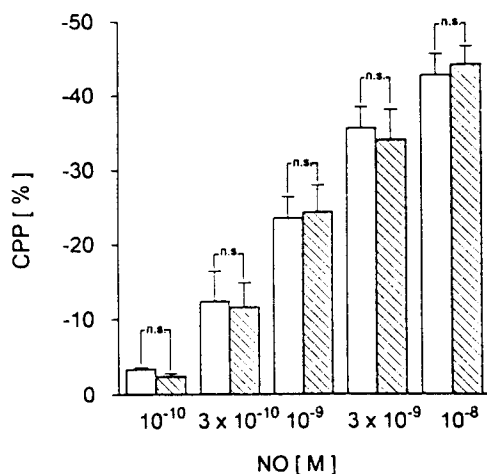


Fig 1. Bar graph shows concentration-response curve for coronary vasodilation elicited by authentic nitric oxide (NO). Isotonic aqueous NO solutions were infused into the coronary circulation of isolated, constant flow-perfused hearts from spontaneously hypertensive rats (SHR, hatched bars) and normotensive Wistar-Kyoto (WKY) rats (open bars) at a rate of 50 to 200  $\mu$ L/min. Changes in coronary perfusion pressure (CPP) are expressed as percent from control; n.s. indicates no significant difference between SHR and WKY (n=5-7).

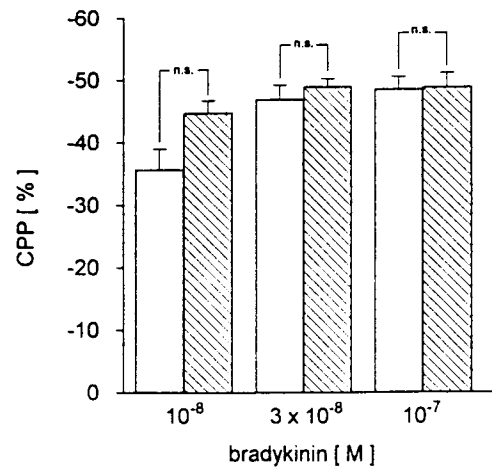


Fig 2. Bar graph shows bradykinin-induced coronary vasodilation in isolated, constant flow-perfused hearts from spontaneously hypertensive rats (SHR, hatched bars) and normotensive Wistar-Kyoto (WKY) rats (open bars). Changes in coronary perfusion pressure (CPP) in percent from control are plotted vs the respective bradykinin concentration; n.s. indicates no significant differences between SHR and WKY (n=6-8).

### Bradykinin-Induced NO Release

Bradykinin significantly decreased CPP from control in the WKY and SHR groups at each concentration ( $10^{-8}$  to  $10^{-7}$  mol/L). The bradykinin-induced coronary vasodilation, as estimated from the absolute reduction in CPP, was significantly larger in SHR than in WKY hearts ( $-33 \pm 4$  versus  $-56 \pm 4$  mm Hg at  $10^{-8}$  mol/L,  $-44 \pm 3$  versus  $-60 \pm 5$  mm Hg at  $3 \times 10^{-8}$  mol/L, and  $-46 \pm 4$  versus  $-60 \pm 7$  mm Hg at  $10^{-7}$  mol/L bradykinin for WKY and SHR hearts, respectively). However, when the data are expressed as percent changes from control CPP, results were not significantly different between SHR and WKY hearts (see Fig 2). Consequently, the decrease in CVR observed on bradykinin infusion was larger in the SHR compared with the WKY group, whereas percent changes from control were comparable in both groups ( $-51\%$  and  $-50\%$ , WKY and SHR, respectively; see Table 2). Moreover, CVR normalized to heart weight (CVR<sub>g</sub>) was comparable in both groups.

In the same hearts, NO release into the coronary circulation was measured during bradykinin infusion. Bradykinin increased NO release from isolated WKY and SHR hearts in a concentration-dependent manner. The maximal rates were similar ( $161 \pm 21$  and  $169 \pm 45$  pmol/min, WKY versus SHR, respectively). Although NO release from isolated SHR hearts tended to be higher, differences between groups did not reach statistical significance (see Fig 3A). Since the wet heart weight was approximately 30% higher in SHR compared with WKY hearts, the surface of the coronary endothelium that contributes to the total amount of released NO might be higher. Therefore, data for NO release were normalized to organ wet weight, still revealing comparable values for NO release in both groups (see Fig 3B). At a bradykinin concentration of  $10^{-7}$  mol/L, NO formation increased by  $155 \pm 20$  and  $130 \pm 35$  pmol/min  $\times$  g over basal release (WKY and SHR, respectively).

To evaluate whether the NO concentrations determined in the coronary circulation were sufficient to account for the bradykinin-induced coronary vasodilation, we compared the concentration-response curves of exogenously

TABLE 2. Bradykinin-Induced Changes in Coronary Vascular Resistance

Drug Infusion	CVR, mm Hg · min/mL		CVR <sub>g</sub> , mm Hg · min/mL · g	
	WKY	SHR	WKY	SHR
Control	6.4±0.2	8.6±0.2*	6.1±0.2	6.6±0.6
Bradykinin, 10 <sup>-8</sup> mol/L	4.2±0.2†	4.9±0.3*	4.1±0.2†	3.8±0.3
Bradykinin, 3×10 <sup>-8</sup> mol/L	3.5±0.1†	4.4±0.2*	3.4±0.1†	3.4±0.1
Bradykinin, 10 <sup>-7</sup> mol/L	3.2±0.1†	4.3±0.1*	3.0±0.1†	3.3±0.1

WKY indicates Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; CVR, coronary vascular resistance; and CVR<sub>g</sub>, CVR normalized to heart weight. n=6-8.

\*P<.05, WKY vs SHR.

†P<.05, control vs bradykinin.

applied and endogenously formed NO. As can be seen from Fig 4, the amounts of intracoronarily formed NO after bradykinin infusion were within the vasodilator range of exogenously applied NO and can thus readily explain the observed coronary vasodilation. In addition, the slightly higher degree of coronary vasodilation observed on infusion of exogenous NO in SHR hearts was paralleled by a slightly higher NO concentration achieved with intracoronarily applied bradykinin.

### Effects of Basal NO Release on CVR

Fig 5 shows data for the basal release of NO into the coronary circulation observed in WKY and SHR hearts. Basal NO release was approximately 91% higher in SHR compared with WKY hearts ( $P<.05$ ). This difference was smaller after normalization for heart wet weight (50%) but remained statistically significant (see Fig 5B).

To test for the possible physiological and pathophysiological importance of an elevated basal NO release in SHR hearts, we performed a separate set of experiments in which L-NAME, a specific inhibitor of the NO synthase, was infused intracoronarily. At a final concentration of 10<sup>-4</sup> mol/L, L-NAME significantly increased CPP in both groups. L-NAME-induced coronary vasoconstriction was significantly more pronounced in SHR hearts compared with WKY hearts (25±3 versus 12±2 mm Hg). When expressed as percent changes from control, increases in CPP were still significantly higher in SHR (23±1%) than in WKY (14±2%) hearts. Consequently, CVR on infusion of L-NAME, either in absolute values or normalized to heart weight, was significantly higher in SHR compared with WKY hearts (see Table 3).

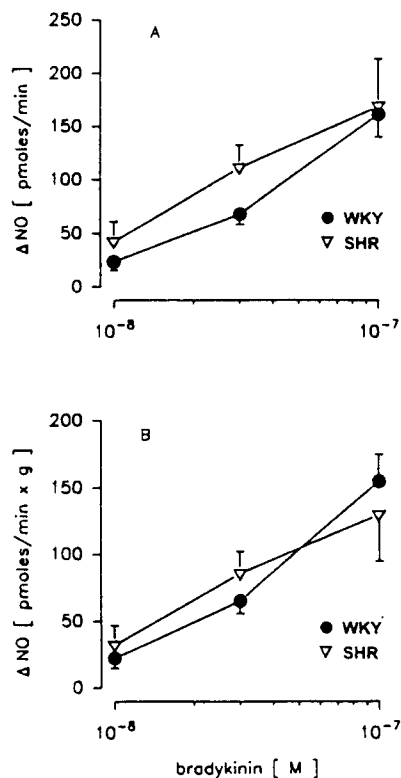


FIG 3. Line graphs show effects of bradykinin on nitric oxide (NO) release into the coronary circulation of isolated, constant flow-perfused hearts from spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats. A, Absolute values for the rate of NO release elicited by bradykinin; B, rate of NO release after normalization to heart wet weight (n=5).

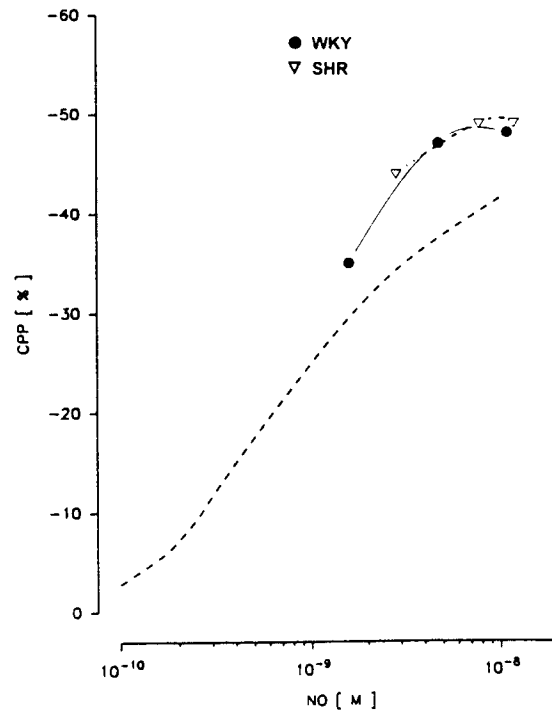


FIG 4. Plot shows comparison of the effect of exogenously applied and endogenously formed nitric oxide (NO) on coronary perfusion pressure (CPP) in isolated, constant flow-perfused hearts from spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats. Dashed line represents the concentration-response curve for the vasodilator effect of exogenously applied NO (see Fig 1). Final concentrations of endogenously formed NO measured on application of different bradykinin concentrations are plotted against the respective changes in CPP (data are from Figs 2 and 3).

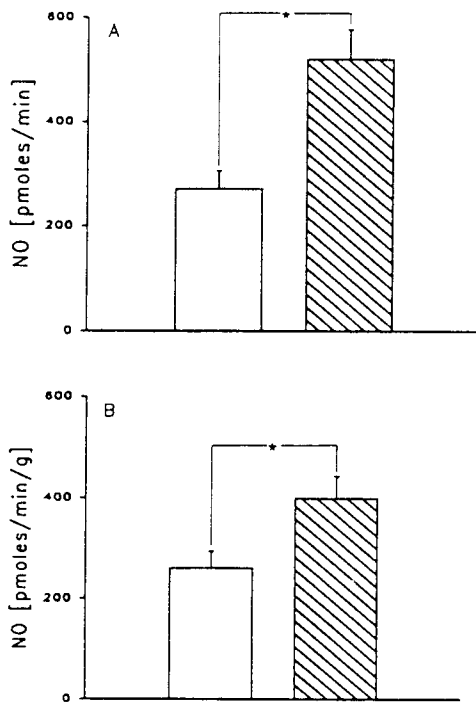


Fig 5. Bar graphs show basal release of nitric oxide (NO) into the coronary circulation of isolated, constant flow-perfused hearts from spontaneously hypertensive rats (SHR, hatched bars) and normotensive Wistar-Kyoto (WKY) rats (open bars). A, Absolute values for the rate of NO release; B, rate of NO release after normalization to heart wet weight. \*Significant differences between SHR and WKY (each n=5).

We also investigated the effects of L-NAME on basal NO release. L-NAME reduced the basal NO release in WKY hearts by 119 pmol/min and in SHR hearts by 117 pmol/min (see Fig 6A). Thus, although the absolute amount of reduction in NO release by L-NAME was comparable in both groups, the relative degree of the percent inhibition of NO release from control was significantly higher in WKY compared with SHR hearts (-44% versus -22%, respectively). This relation remained the same after data were normalized to heart wet weight, as depicted in Fig 6B.

Fig 7 depicts the relation between CPP and NO release under control conditions for individual WKY and SHR hearts. As described before, the average NO release and average CPP were less in the WKY group. Most importantly, close inverse correlations between both parameters were uncovered within each group. The

TABLE 3. Effects of L-NAME on Coronary Perfusion Pressure and Vascular Resistance

Parameter	WKY	SHR
CPP, mm Hg		
Control	81±2	110±5*
L-NAME	93±4†	134±7†
CVR, mm Hg · min/mL		
Control	5.8±0.2	7.8±0.4*
L-NAME	6.6±0.3†	9.5±0.7†
CVR <sub>g</sub> , mm Hg · min/mL · g		
Control	5.5±0.2	6.0±0.6
L-NAME	6.3±0.1†	7.3±0.1†

L-NAME indicates N<sup>G</sup>-nitro-L-arginine methyl ester (10<sup>-4</sup> mol/L); CPP, coronary perfusion pressure; and definitions as in Table 2. n=3-4.

\*P<.05, WKY vs SHR.  
†P<.05, control vs L-NAME.

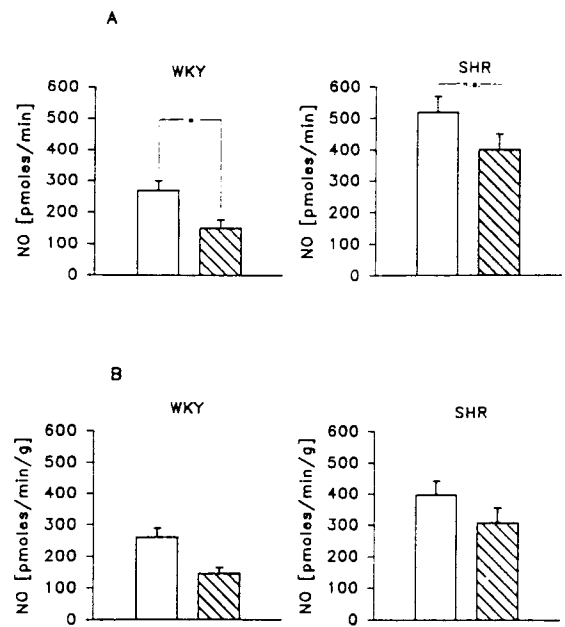


Fig 6. Bar graphs show effects of N<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME) (10<sup>-4</sup> mol/L) on basal nitric oxide (NO) formation in isolated, constant flow-perfused hearts from spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats. A, Absolute values for the rate of NO release; B, rates of NO release after normalization to heart wet weight. \*Significant differences between SHR and WKY; n.s. indicates no significant differences (n=3-4). Open bars, control; hatched bars, L-NAME.

higher the NO release, the lower the CPP. Comparable results were obtained when CVR and basal NO formation were normalized for heart weight, as can be seen in Fig 7.

### Discussion

The major results of the present study are as follows: (1) Coronary vascular smooth muscle of normotensive and hypertensive rats expressed a similar sensitivity toward exogenously applied NO. (2) Bradykinin-induced changes in NO release were comparable in both groups, and the amount of released NO on stimulation with bradykinin was sufficient to account for the observed coronary vasodilation. (3) The higher level of CVR observed in SHR hearts was associated with a significantly elevated rate of basal NO formation. (4) A close inverse relation exists between CVR and basal NO formation within both groups. Collectively, NO synthesis and release into the coronary circulation from SHR hearts is not specifically impaired but rather preserved and critically determines CVR.

During the development of arterial hypertension, several functional and morphological alterations of the vascular endothelium and smooth muscle cells have been described (for review, see References 16 and 17). Various experimental and clinical studies designed to estimate the pathophysiological importance of NO for the regulation of vessel tone in arterial hypertension have revealed conflicting results<sup>18-20</sup> (for review, see Reference 21). Current data in the literature do not allow the conclusion that a reduced NO synthesis is the major cause for an increased vascular resistance in arterial hypertension. Most of these functional studies used inhibitors of NO synthase or acetylcholine stimulation to indirectly assess endothelium-dependent vaso-

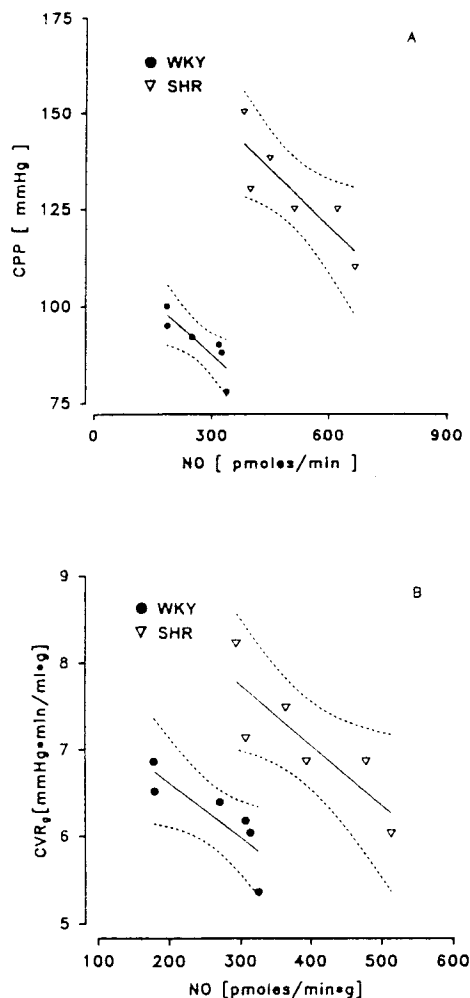


FIG 7. Plots show relation between basal coronary vascular tone and nitric oxide (NO) formation in isolated, constant flow-perfused hearts from spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) rats. A, Linear regression analysis for basal NO release and coronary perfusion pressure (CPP). WKY:  $y = -0.090x + 114.6$ ,  $r = -0.856$ ,  $r^2 = 73.2\%$ ,  $P = .030$ ; SHR:  $y = -0.097x + 178.9$ ,  $r = -0.849$ ,  $r^2 = 72.1\%$ ,  $P = .032$  (n=6). B, Linear regression analysis for basal NO formation and coronary vascular resistance normalized to heart wet weight (CVR<sub>g</sub>). WKY:  $y = -0.006x + 7.87$ ,  $r = -0.844$ ,  $r^2 = 71.2\%$ ,  $P = .035$ ; SHR:  $y = -0.007x + 9.82$ ,  $r = -0.841$ ,  $r^2 = 70.7\%$ ,  $P = .036$  (n=6). Dashed lines represent 95% confidence limits.

motion rather than quantifying NO directly. This direct quantification of NO, however, is crucial to establish a causal relation between a reduced NO production and the blunted endothelium-dependent vasomotion observed in arterial hypertension. The present study is apparently the first that has quantified NO release into the coronary circulation of hypertensive animals.

#### Effects of Authentic NO on Coronary Hemodynamics

The degree of maximal coronary vasodilation observed after NO infusion and the low threshold concentration of approximately 1 nmol/L demonstrate that NO is a powerful dilator of coronary resistance vessels. The present data clearly indicate that the responsiveness of coronary vascular smooth muscle to exogenous NO is similar in hearts from normotensive and hypertensive rats. Therefore, an uncoupling of the vascular endothelial and smooth muscle compartment by an impaired

diffusion of NO secondary to an intimal thickening is considered unlikely to represent one pathomechanism for the endothelial dysfunction observed in arterial hypertension.<sup>12</sup> Superoxide anion radicals, which have been demonstrated to be produced by vascular endothelial cells, may react rapidly with NO to form peroxynitrite, which decomposes to vasoinactive nitrate and thus has been considered as an endothelium-dependent "constricting factor" counteracting the effect of EDRF/NO.<sup>11,22,23</sup> In the present model of arterial hypertension, however, this interaction appears not to be important for the regulation of coronary vessel tone, as the dose-response curves for the NO-induced drop in CPP were superimposable in both groups.

#### Effects of Bradykinin on Coronary Hemodynamics and NO Release

In the present study, bradykinin rather than acetylcholine, serotonin, or histamine was chosen to stimulate endothelial NO release because bradykinin is the more powerful stimulus for NO synthesis in the coronary circulation,<sup>7</sup> and at the concentrations used, it exerts its action specifically at the endothelium and not at the vascular smooth muscle.<sup>24</sup> As the perfusion medium was supplemented with indomethacin, effects of prostacyclin on CPP have been ruled out. Both the degree of coronary vasodilation elicited by bradykinin and the extent of NO formation were slightly higher in SHR compared with WKY hearts. Because of its radical nature, NO is considered to readily pass cellular membranes. Thus, it appears likely that the amounts of NO released to either the luminal or abluminal site of the endothelium are comparable. The present setup does not allow us to conclude that the portion of NO detected photometrically at the luminal site matches largely the portion of abluminally released vasoactive NO. However, we can conclude that in both groups the amount of endogenously formed NO was at least within the vasodilator effective range of authentic NO and can thus account for the bradykinin-induced coronary vasodilation (see Fig 4). At present, no data are available in the literature on hypertension-associated alterations of endothelium-dependent NO release after stimulation with bradykinin in the coronary vasculature. However, Cachofeiro and Nasjletti<sup>25</sup> recently reported that in isolated perfused kidneys from SHR, WKY rats, and angiotensin II-treated Sprague-Dawley rats, the vasodilator response to bradykinin was nearly equal in each group. This finding is in line with the present data and shows that the degree of bradykinin-induced vasodilation is independent of the rat strain used. This furthermore weakens the hypothesis that an impaired NO-dependent vasodilation is responsible for the higher total vascular resistance in hypertension.

#### Basal Release of NO

Resting and minimal CVR might be affected differently in the hypertensive heart. Therefore, we further quantified basal NO release in SHR and WKY hearts. In this study, heart wet weight in 16- to 20-week-old SHR was approximately 30% higher compared with that of the normotensive control strain. Thus, an increased extravascular pressure due to myocardial hypertrophy and/or concomitant interstitial fibrosis may have contributed to the elevated resting CVR. However, even after CVR was normalized to heart wet weight, CVR<sub>g</sub> values

were still significantly higher in SHR. This indicates that other factors within the coronary vasculature, such as functional changes in endothelial and vascular smooth muscle cells or morphological changes such as rarefaction of the microvasculature or an increased wall-to-lumen ratio, must contribute to the increased coronary resistance.<sup>26</sup> Surprisingly, the elevated  $CVR_g$  in SHR was accompanied by a 50% increase of NO release into the coronary circulation. Therefore, the increased resting tone of coronary vessels cannot be attributed to an impaired NO synthesis. In line with this interpretation are recently reported data from Arnal et al<sup>27</sup> at the level of the conduit arteries. In that study, the cyclic GMP content in the aortic wall, as a second messenger of NO, was not significantly different under control conditions and after administration of L-NAME between WKY rats and SHR. The authors concluded that basal release of NO does not appear to be impaired in SHR, at least in conduit arteries, but represents a major counterregulatory mechanism for the increased vascular resistance in this genetic model of arterial hypertension.<sup>27</sup> Similar observations were reported in a very recent study<sup>28</sup> in which basal NO synthase activity, determined by conversion of radiolabeled L-arginine to L-citrulline, in aortic segments from SHR was twice that of segments from the normotensive control strain, probably as a compensatory mechanism to hypertension at least at the level of conduit arteries.

Rising perfusion flow and shear stress are regarded as major stimuli for the release of basal EDRF/NO.<sup>29,30</sup> The experimental setup used in the present study does not allow the measurement of shear stress within the coronary circulation. Because of differences in heart weight, the constant flow perfusion with 14 mL/min resulted in flow rates of 13.5 and 10.8 mL/min $\times$ g in WKY and SHR hearts, respectively. It thus appears reasonable that shear stress elicited by saline perfusion medium was higher in the coronary circulation of isolated WKY hearts than in SHR hearts. However, the major determinant of shear stress acting on the luminal site of the endothelium is the vascular tone and thus the height of CVR. Therefore, the elevated CVR, which was more than 30% higher in SHR compared with WKY hearts, may explain the enhanced basal NO formation observed in SHR hearts and may serve the purpose of compensating for increased CVR.

Because of the limited oxygen transport capacity of saline perfusion medium compared with blood, the chosen flow rate is almost 10-fold higher than the prevailing basal coronary blood flow *in vivo*. This in turn may have resulted in a severalfold higher shear stress at the coronary endothelium in both groups and thus may have contributed in part to the rather high level of basal NO formation compared with the relatively small additional increase in NO formation seen on stimulation with bradykinin. However, the comparison of the sensitivity of coronary vascular smooth muscle with authentic NO and endogenously formed NO on infusion of bradykinin (see Fig 4) will not have been affected by this inherent methodological feature of isolated, saline-perfused hearts, as the rate of basal NO formation was comparable in both experimental groups because of similar perfusion conditions.

The maintenance of flow despite changes in pressure during constant myocardial oxygen demand is considered as autoregulation of coronary blood flow.<sup>31</sup> In the

canine and human coronary vasculature of hypertrophied ventricles due to arterial hypertension, it has been demonstrated that the lower and upper limits of the autoregulatory range are shifted to higher values.<sup>32,33</sup> To test whether the relation between CPP and NO production is changed in SHR, we plotted CPP and  $CVR_g$  versus NO release (Fig 7). Two interesting features were revealed: (1) Within each group, resting CPP and  $CVR_g$  increased with decreasing basal NO release, indicating that NO participates in setting the resting tone of resistance vessels of normotensive and hypertensive rat hearts. (2) The relation of CPP and NO release is shifted to higher levels in SHR compared with WKY hearts. This suggests that the increased rate of NO release in SHR hearts helps to maintain CPP closer to physiological levels.

In the present experimental model, the basal NO formation within the coronary circulation significantly determines the resting tone of coronary resistance vessels, as indicated by the coronary vasoconstriction observed on application of L-NAME. Interestingly, L-NAME reduced basal NO release equally effectively in both groups by almost 120 pmol/min, irrespective of the level of basal NO formation, although CPP and  $CVR_g$  increased more in SHR compared with WKY hearts. This might indicate that not the absolute level of basal NO formation *per se* but the relation between CPP and NO release is the critical factor in determining the degree of coronary vasoconstriction. Since L-arginine is present in mammalian cells at concentrations of approximately 0.1 to 1 mmol/L, the L-NAME concentrations used may not have been sufficiently high enough to ensure effective competitive blockade of NO synthase. Unfortunately, L-NAME in concentrations higher than  $10^{-4}$  mol/L interferes with the hemoglobin assay because of its absorbance characteristics, precluding an investigation of its effects on NO formation at higher concentrations. The present data indicate that the hemodynamic response to an inhibitor of NO synthase does not allow one to derive conclusions about the extent of reduction of basal NO release in blood vessels from hypertensive rats or patients without parallel quantification of NO formation. Therefore, the simultaneous determination of NO release and hemodynamic responses appears to be mandatory, and efforts should be directed toward the measurement of this important vasodilator under *in situ* conditions.

### Study Limitations and Future Directions

Although the SHR and its normotensive WKY control strain represent a widely accepted experimental model for genetically determined arterial hypertension, species differences in NO release compared with other models—such as deoxycorticosterone acetate hypertensive rats; one-kidney, one clip rats; and renin-, angiotensin-, or coarctation-induced hypertension—cannot be ruled out by the present study. Thus, data derived from experiments with any of these animal models of arterial hypertension should be extrapolated to hypertensive patients only with great caution. Nevertheless, the main result of the present study shows clearly that increased vascular resistance is not necessarily linked to decreased NO formation. On the contrary, basal NO formation rather can be enhanced, at least in the coronary circulation of SHR, probably as a result of increased shear stress caused by elevated CVR. However, the results

from the present study do not allow one to decide whether or not the elevated basal release of NO actually represents a compensatory reflex mechanism that may help to compensate for the elevated CVR in hypertensive hearts during the course of hypertension development. To address this question, long-term studies in 10- to 60-week-old SHR and WKY rats are highly desirable.

The present study emphasizes the fact that a method for NO quantification under in vivo conditions appears not only desirable but mandatory to assess the pathophysiological significance of NO in arterial hypertension in humans. Because of the extremely rapid metabolism of NO, which is converted to nitrite in human plasma and consecutively oxidized to nitrate within the erythrocytes,<sup>34</sup> this aim remains presently unresolved. The oxyhemoglobin assay used in the present study offers the advantage of an on-line detection system for picomolar amounts of NO at its site of release in intact saline-perfused organ preparations. In the future, quantification of NO in vivo via parallel detection of electrochemical activity<sup>35</sup> and nitrite<sup>34</sup> or nitrosothiols<sup>36</sup> as specific indicators of the constitutive NO synthase might represent a promising strategy to resolve this issue.

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