Antihypertensive Therapy Augments Endothelium-Dependent Relaxations in Coronary Arteries of Spontaneously Hypertensive Rats

Marcel R. Tschudi, MSc; Leoluca Criscione, PhD; Dragutin Novosel, MD; Karl Pfeiffer; Thomas F. Lüscher, MD

Background Coronary artery disease is an important complication of hypertension. Therefore, the effects of antihypertensive therapy on the endothelial nitric oxide (NO)/L-arginine pathway and vascular smooth muscle were studied in left anterior descending coronary arteries of Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHR). Angiotensin II (AT1) receptor antagonists CGP 48369 and valsartan, angiotensin-converting enzyme inhibitor benazepril HCl, and calcium antagonist nifedipine were used as antihypertensive agents.

Methods and Results Rings were examined in myograph systems for isometric tension recording. In untreated WKY and SHR rings, acetylcholine (10^-9 to 10^-5 mol/L) but not bradykinin, substance P (both 10^-6 mol/L), or thrombin (1 U/mL) induced comparable endothelium-dependent relaxations. These relaxations were markedly decreased by NO^-monomethyl-L-arginine (10^-4 mol/L) and fully prevented by N^-nitro-L-arginine methyl ester (10^-4 mol/L) or methylene blue (10^-5 mol/L). In vitro treatment of WKY and SHR rings with benazeprilat, CGP 48369, or valsartan (3 x 10^-7 mol/L) did not affect responses to acetylcholine. In SHR, chronic therapy for 8 weeks with benazepril HCl, CGP 48369, valsartan, or nifedipine (each 10 mg · kg^-1 · d^-1 PO) similarly reduced blood pressure and increased endothelium-dependent relaxations to acetylcholine (log shift at IC50, ie, half-maximal inhibition of a preceding contraction, 10^-6, 10^-5, and 10^-4 mol/L, main difference: P<.05 versus control), whereas relaxations to NO donor 3-morpholino sydnonimine (SIN-1, 10^-4 to 10^-3 mol/L) remained unaffected. In WKY, chronic therapy with nifedipine (10 mg · kg^-1 · d^-1 PO) affected neither blood pressure nor relaxations to acetylcholine or SIN-1.

Conclusions In rat coronary arteries, NO is synthesized via the endothelial L-arginine pathway and released after stimulation with acetylcholine. In SHR, chronic antihypertensive therapy with either angiotensin receptor antagonists, an angiotensin-converting enzyme inhibitor, or a calcium antagonist specifically increased the normal endothelium-dependent relaxations to acetylcholine, probably because of their blood pressure-lowering effects, whereas the responsiveness of vascular smooth muscle to NO remained unaffected. (Circulation. 1994;89:2212-2218.)

Key Words • benazepril • CGP 48369 • methylene blue • arginine • nifedipine • valsartan

Endothelial cells are a source of relaxing factors that can profoundly affect vascular tone. An important relaxing factor is nitric oxide.1-3 Besides acetylcholine, many other vasodilators or physical stimuli trigger endothelium-dependent relaxations in arteries of different species, including humans.4-5 The endothelium also can reduce the effects of vasoconstrictor substances through basal release of endothelium-derived nitric oxide.1,6 In cultured cells stimulated with bradykinin, nitric oxide is formed from the amino acid L-arginine.2 The endothelial L-arginine pathway can be inhibited by analogues of the amino acid such as N^-nitro-L-arginine methyl ester (L-NAME) or N^-monomethyl-L-arginine (L-NMMA).7

Hypertension is associated with decreased endothelium-dependent relaxations.8-13 This has been demonstrated not only in large conduit arteries but also in resistance arteries but also obtained from hypertensive rats, as well as in the human forearm and coronary circulation of hypertensive patients.19-21 In the aorta of hypertensive rats, antihypertensive treatment with reserpine, hydrochlorothiazide, and hydralazine improves endothelial function, indicating that endothelial dysfunction in hypertension is reversible, at least in the aorta.22

In recent years, several new classes of antihypertensive drugs such as angiotensin-converting enzyme inhibitors and calcium antagonists have been developed,23-26 and more recently, orally active, nonpeptide angiotensin II receptor antagonists (ie, losartan27 and valsartan28). Angiotensin II receptor antagonists have antihypertensive properties and may represent more effective inhibitors of the renin-angiotensin system than angiotensin-converting enzyme inhibitors.29

The present study was designed to investigate the effects of chronic antihypertensive therapy with the novel, nonpeptide angiotensin II (AT1) receptor antagonists CGP 48369 and valsartan compared with those of a converting enzyme inhibitor or calcium antagonist on the endothelial L-arginine pathway and vascular smooth muscle function in the coronary circulation of normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats.
## Methods

### Experimental Animals

All experiments were performed in 15-week-old normotensive Wistar-Kyoto rats (WKY) or spontaneously hypertensive rats (SHR; IFFA CREDO). For a small series of experiments, WKY and SHR rats from a different supplier were also investigated (Charles River Wiga GmbH). To evaluate the effects of antihypertensive therapy, WKY and SHR were obtained at 6 weeks of age and maintained on standard rat chow with free access to drinking water. At the age of 7 weeks, WKY were assigned to a control group or nifedipine and SHR to one of four treatment groups (benazepril, CGP 48369, valsartan, or nifedipine). Drug-treated rats were treated orally by gavage with benazepril HCl, CGP 48369, valsartan, or nifedipine (each 10 mg · kg⁻¹ · d⁻¹) for 8 weeks. The medication was placed directly into the stomach by daily oral insertion of a tube. Control rats received only a solvent. The experimenter (M.R.T.) was unaware of the treatment assigned to the studied rats. On the day of the experiment (age 15 weeks), blood pressure was measured 18 to 20 hours after administration of the drugs in conscious rats by the tail-cuff method (Table 1). Then rats were anesthetized with pentobarbital (40 mg/kg IP), and the heart was removed and placed into cold (4°C) Krebs-Ringer bicarbonate solution (mmol/L): NaCl 118.6, KCl 4.8, CaCl₂ 2.5, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25.1, edetate calcium disodium 0.026, glucose 10.1 (control solution).

### Experimental Setup

Left anterior descending coronary arteries were dissected free under a microscope (Wild-Leitz). Rings (1.8 to 2 mm in length; internal diameter, 300 μm) were mounted in a modified version of the myograph system described by Mulvany and Halpern for measurement of isometric force. Organ chambers were filled with 12.5 mL of control solution (37°C, 95% O₂/5% CO₂). Rings were held in place by means of two stiff tungsten wires (diameters, 30 and 50 μm) that were carefully passed through the lumen and fastened to clamps attached to a force transducer (Showa Sokki LB-5, Rikadenki) and to a micromanipulator (Narishige) for adjustment of muscle length. The isometric force-to-length relation to each ring was determined by repeated exposures to 100 mmol/L KCl at increasing levels of vessel length. The vessels were then held at the optimal point of the length-to-tension relation at which maximum active force was produced for all subsequent experiments (200±8 mg).

### Protocols

The relaxing effects of acetylcholine and 3-morpholinosydnonimine (SIN-1) were studied by adding increasing concentrations of the drugs (10⁻⁵ to 10⁻³ mol/L) on top of a contraction to serotonin (10⁻⁴ mol/L). The acute effects of indomethacin (10⁻⁵ mol/L), L-NAME (10⁻⁴ mol/L), L-NMMA (10⁻⁴ mol/L), methylene blue (10⁻⁵ mol/L), benazepril (3×10⁻⁷ mol/L), CGP 48369 (3×10⁻⁷ mol/L), and valsartan (3×10⁻⁷ mol/L) on acetylcholine-induced relaxations were tested by adding increasing concentrations of acetylcholine on top of a contraction evoked by serotonin (10⁻⁶ mol/L) before and after incubation with these drugs for 30 minutes. To study contractions evoked by serotonin (10⁻⁷ to 10⁻⁴ mol/L), increasing concentrations of the monoamine were added in a cumulative fashion to quiescent preparations and compared with the contraction induced by KCl (100 mmol/L).

### Drugs

The following drugs were used (all from Sigma Chemical Co unless otherwise stated): acetylcholine hydrochloride, bradylkinin, indomethacin, L-NAME, L-NMMA, methylene blue, pentobarbital (Abbott), 5-hydroxytryptamine creatinine sulfate (serotonin; Serva), SIN-1 (Hoechst Pharmaceuticals), substance P, and thrombin. All concentrations of the drugs used in vitro are expressed as final molar concentration in the organ chambers. Benazepril HCl, benazepril active form, CGP 48369, valsartan, and nifedipine were synthesized at the Chemistry Department, CIBA-GEIGY, Basel, Switzerland.

### Calculations and Statistical Analysis

For statistical analysis, the concentration causing half-maximal contraction (EC₅₀) or half-maximal inhibition of a preceding contraction (IC₅₀), the maximal relaxation (percent), and the area under the concentration-response curve (arbitrary units) were calculated for each experiment. EC₅₀ and IC₅₀ were expressed as negative log molar (pD₂) value. Data are given as mean±SEM. In each set of experiments, n equals the number of animals studied. Statistical evaluation was done by unpaired Student’s t test or by ANOVA followed by Scheffe’s F test. Means were considered significantly different at values of P<.05.

### Results

#### L-Arginine Pathway in Rat Coronary Arteries

In rat coronary arteries with endothelium, acetylcholine (10⁻⁴ to 3×10⁻⁴ mol/L) caused potent relaxations that were absent after removal of the endothelium and fully prevented by the inhibitor of nitric oxide formation L-NAME (10⁻⁴ mol/L) or the inhibitor of soluble guanylyl cyclase methylene blue (10⁻⁴ mol/L, P<0.01, Fig 1). L-NMMA (10⁻⁴ mol/L), another inhibitor of nitric oxide formation, was less potent than L-NAME (10⁻⁴ mol/L) but also markedly reduced the maximal response to acetylcholine from 87±4% to 51±9% and significantly shifted the concentration-response curve to the right (log shift at IC₅₀, 4.3-fold versus control;
P<.05, n=6). In contrast, the cyclooxygenase inhibitor indomethacin (10^{-6} mol/L) did not affect the response to acetylcholine in WKY or SHR rats (n=3, data not shown).

Other agonists of the L-arginine pathway such as bradykinin (10^{-6} mol/L; n=3), substance P (10^{-6} mol/L; n=3), or thrombin (1 U/mL; n=3) were ineffective in this preparation (Fig 2).

**Relaxations to Acetylcholine in WKY and SHR Coronary Arteries**

In coronary arteries obtained from untreated WKY or SHR, endothelium-dependent relaxations to acetylcholine (10^{-9} to 10^{-5} mol/L) were comparable. The maximal relaxation was 78±7% in WKY and 77±5% in SHR, and the IC_{50} values were 6.7±0.3 and 6.2±0.2, respectively (NS, n=4). Similar results were obtained in WKY and SHR rats obtained from a different supplier (n=6, data not shown; NS both for n=6 and pooled data, n=10).

**Acute Effect of Antihypertensive Drugs**

In WKY and SHR, in vitro treatment of coronary rings with either benazepril, CGP 48369, or valsartan (each 3x10^{-7} mol/L) had no acute effects on endothelium-dependent relaxations to acetylcholine (Table 1, n=5). The effect of nifedipine (3x10^{-7} mol/L) on the relaxations to acetylcholine could not be tested, because it was impossible to precontract the vessels with serotonin (10^{-4} mol/L) in the presence of nifedipine (n=3, data not shown).

**Effect of Chronic Antihypertensive Therapy**

**Blood Pressure and Body Weight**

At 15 weeks of age, all forms of chronic antihypertensive therapy led to a comparable decrease in systolic blood pressure in SHR that averaged 17 to 23 mm Hg (Table 2, P<.05), although body weight did not differ. In WKY, chronic treatment with nifedipine affected neither blood pressure nor body weight (Table 2).

**Relaxations to Acetylcholine**

In SHR, antihypertensive therapy with either benazepril HCl, CGP 48369, valsartan, or nifedipine (each 10 mg · kg^{-1} · d^{-1} for 8 weeks) significantly increased endothelium-dependent relaxations evoked by acetylcholine (10^{-9} to 10^{-5} mol/L, Fig 3, left) to a similar degree. The log shift of the concentration-response curve at IC_{50} averaged 10-, 8-, 13-, and 13-fold in rats treated with benazepril HCl, CGP 48369, valsartan, or
Relaxations to SIN-1

The relaxations to the nitric oxide donor SIN-1 (10^−9 to 10^−3 mol/L) were identical in untreated and treated SHR (n=4, Fig 3, right) and WKY. The IC_{50} values, the maximal responses, and the areas under the concentration-response curve of the relaxations to SIN-1 in SHR and WKY are listed in Table 3.

Contractions to Serotonin

In SHR and WKY, the contractions induced by serotonin (10^−9 to 10^−4 mol/L) were unaffected by the various antihypertensive regimens used (for SHR, Fig 4, n=4) and did not statistically differ from each other in the two strains of rats. The EC_{50} values, the maximal responses, and the areas under the concentration-response curve of the contractions to serotonin in SHR and WKY are given in Table 4.

Discussion

This study demonstrates that in SHR, antihypertensive therapy with the novel, nonpeptide angiotensin II (AT_{1}) receptor antagonists valsartan and CGP 48369 led to a significant decrease in systolic blood pressure and a pronounced increase of endothelium-dependent relaxations induced by acetylcholine comparable to the effects obtained with a converting enzyme inhibitor such as benazepril HCl or the calcium antagonist nifedipine.

Although nitric oxide has been identified as the endogenous nitrovasodilator formed within endothelial cells from L-arginine, debate continues as to whether...
fully prevented by the inhibitor of nitric oxide formation L-NAME or the inhibitor of soluble guanylyl cyclase methylene blue and markedly reduced by L-NMMA. Since indomethacin (to inhibit the formation of prostaglandins) did not affect relaxations, it appears that, in contrast to the aorta and renal artery, acetylcholine does not stimulate the formation of the cyclooxygenase-dependent endothelium-derived contracting factor (ie, prostaglandin H₂ or thrombin) in epicardial coronary arteries of WKY or SHR. Interestingly, and in contrast to porcine coronary arteries, other endothelium-dependent vasodilators such as bradykinin, substance P, and thrombin were ineffective in rat coronary arteries, indicating that a considerable heterogeneity in the expression of endothelial receptors exists in the coronary circulation of different species.

Normally, hypertension is associated with decreased endothelium-dependent relaxations not only in large conduit arteries but also in resistance arteries as well as in the forearm and coronary circulation of hypertensive patients. In coronary arteries of SHR, however, endothelium-dependent relaxations to acetylcholine were comparable to those in normotensive WKY. Similar results were also obtained in WKY and SHR rats from a different supplier. Furthermore, similar observations were made in our previous studies in rat coronary arteries as well as in renal arteries of SHR and WKY rats. Thus, the endothelium of adult SHR coronary arteries has an ability to modulate coronary vascular tone through the release of endothelium-derived nitric oxide comparable to that of age-matched WKY, at least up to the age of 15 weeks. It remains possible that in the SHR, the endothelial defect up to this age involves primarily the intraluminal surface of the hypertensive endothelium, and such a subtle alteration may not be detected in ring preparations in which agonist reaches both the luminal and abluminal side of the blood vessel wall. More likely, however, WKY may not represent an optimal control for studies in the SHR coronary circulation, and the SHR actually would show impaired endothelial function if they were compared with their own strains in the absence of hypertension, for instance during antihypertensive therapy (see below).

In WKY and SHR, in vitro treatment of coronary rings with either benazepril, CGP 48369, or valsartan had no acute effect on endothelium-dependent relaxations to acetylcholine. This indicates that, although a local vascular renin-angiotensin system exists, its activity does not interfere with endothelium-dependent relaxations of rat coronary arteries in either normotension or spontaneous hypertension. The acute effect of nifedipine on relaxations to acetylcholine could not be tested, because it was impossible to precontract coronary arteries with serotonin in the presence of the drug.

Chronic antihypertensive therapy was initiated at an early age (ie, 7 weeks), when blood pressure in the SHR differed only little from WKY. Also, the duration of therapy was prolonged (8 weeks). Both effects may have contributed to the pronounced improvement of endothelial function. Drugs that interfere with the renin-angiotensin system and Ca²⁺ inflow were chosen, because they are effective antihypertensive agents and exert vascular protective effects under certain conditions. Angiotensin receptor antagonists represent a new

![Graph showing lack of effect of chronic antihypertensive therapy in spontaneously hypertensive rats (SHR) with either the nonpeptide angiotensin II receptor antagonists CGP 48369 and valsartan, the angiotensin-converting enzyme inhibitor benazepril HCl, or the calcium antagonist nifedipine on the contractions to serotonin.](image)

**Figure 4.** Graph showing lack of effect of chronic antihypertensive therapy in spontaneously hypertensive rats (SHR) with either the nonpeptide angiotensin II receptor antagonists CGP 48369 and valsartan, the angiotensin-converting enzyme inhibitor benazepril HCl, or the calcium antagonist nifedipine on the contractions to serotonin.

Table 4. Effect of Chronic Antihypertensive Therapy on Sensitivity, Maximal Response, and Area Under the Concentration-Response Curve to Serotonin in Isolated Left Anterior Descending Coronary Arteries of Normotensive Wistar-Kyoto Rats and Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Animals</th>
<th>P₀ Value*</th>
<th>Maximal Response, %†</th>
<th>Area‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated SHR</td>
<td>6.6±2.0</td>
<td>136±2</td>
<td>644±49</td>
</tr>
<tr>
<td>Treated SHR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benazepril HCl</td>
<td>6.9±0.1</td>
<td>135±5</td>
<td>735±21</td>
</tr>
<tr>
<td>CGP 48369</td>
<td>6.7±0.2</td>
<td>143±6</td>
<td>686±54</td>
</tr>
<tr>
<td>Valsartan</td>
<td>6.6±0.1</td>
<td>136±5</td>
<td>658±48</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>6.8±0.2</td>
<td>128±7</td>
<td>674±72</td>
</tr>
<tr>
<td>Untreated WKY</td>
<td>6.6±0.1</td>
<td>116±8</td>
<td>586±47</td>
</tr>
<tr>
<td>Treated WKY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nifedipine</td>
<td>6.2±0.2</td>
<td>89±13</td>
<td>441±64</td>
</tr>
</tbody>
</table>

SHR indicates spontaneously hypertensive rats; WKY, Wistar-Kyoto rats. Data are given as mean±SEM of arteries obtained from four SHR or five WKY animals.

*Negative log EC₅₀ (half-maximal contraction).
†Preceding contraction induced by KCl 100 mmol/L was taken as 100%.
‡Area under the concentration-response curve expressed in arbitrary units (1 to 1000).
therapeutic approach in hypertension that has not yet been compared with angiotensin-converting enzyme inhibitors or calcium antagonists. Under the conditions of this study, all drugs used, ie, the nonpeptide angiotensin receptor antagonists valsartan and CGP 48369, benazepril HCl, and nifedipine, all markedly increased both the sensitivity and maximal response to the endothelium-dependent vasodilator acetylcholine. Since the response to the nitric oxide donor SIN-131,36 and contractions to serotonin were unaltered, this indicates that endothelial function, ie, the production of nitric oxide, was similarly improved by all antihypertensive regimens, whereas vascular smooth muscle function remained unaltered. Since in the SHR, all drugs similarly reduced the rise in arterial blood pressure and led to an identical improvement of endothelial function, blood pressure lowering by itself must be the most important determinant. In line with that interpretation, in WKY, chronic nifedipine therapy did not affect blood pressure or endothelium-dependent relaxations to acetylcholine. These results confirm the above-mentioned hypothesis that in the SHR, high blood pressure does indeed reduce coronary endothelial function.

Coronary artery disease is an important vascular complication of hypertension.47 In most clinical studies, antihypertensive therapy did not affect coronary morbidity and mortality.48-50 A meta-analysis, however, indicates that antihypertensive therapy does reduce coronary artery disease in hypertensive subjects, although less so than cerebrovascular events.51 The results of this experimental study suggest that antihypertensive drugs that interfere with the renin-angiotensin system or calcium influx improve coronary endothelial function, largely because of their blood pressure-lowering effects, provided that therapy is started early. The improvement in endothelial function may be important, because endothelial cells play a protective role in the coronary circulation as inhibitors of platelet function (through the release of nitric oxide and prostacyclin56,57) as well as by preventing coronary vaso- spasm (mainly through the release of nitric oxide in response to platelet-derived products and other vasoconstrictor substances53).

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