Vascular Response to Angiotensin II in Atherosclerosis
Role of the Baroreflex

Klaus Wilfert, Klaus Drischel, Axel Unbehaun, Hans Guski, Pontus B. Persson, Harald M. Stauss

Abstract—High-cholesterol alimentation is associated with an induction of angiotensin-converting enzyme and angiotensin II receptor expression within the vascular wall of the aorta. Despite an enhanced pressure response to angiotensin II in atherosclerotic conscious rabbits, angiotensin II–induced contraction was reduced in isolated vascular rings from the aorta and unchanged in those from the iliac artery. We, therefore, investigated whether cholesterol-induced atherosclerosis enhances overall vascular responsiveness to angiotensin II in intact animals and whether an altered arterial baroreflex sensitivity can explain the discrepancy between experiments in intact animals and isolated blood vessels. Rabbits were maintained on a high-cholesterol diet (2 g/d cholesterol plus 20 mL/d sunflower seed oil, n=11) or on a standard diet (n=12) for 12 weeks. Total serum lipids markedly increased (P<0.05). Tissue examinations 6 weeks after termination of the high-cholesterol diet revealed distinct atherosclerosis and elevated cholesterol content in the aorta (P<0.05). A high-cholesterol diet did not change baseline hemodynamic parameters. However, angiotensin II–induced increases in total peripheral resistance were larger in the atherosclerotic animals (86.3±13.0 versus 41.9±9.7 mm Hg·L⁻¹·min⁻¹, P<0.05). In addition, the blood pressure pulse interval relationship was markedly reduced (slope: 0.80±0.14 versus 0.49±0.06 ms/mm Hg, P<0.05), which suggested that the baroreflex blunted the angiotensin II response to a lesser extent in atherosclerotic animals. In conclusion, the overall vascular responsiveness to angiotensin II is increased in the atherosclerotic conscious rabbit as indicated by the larger increase in total peripheral resistance. An attenuation of the arterial baroreflex sensitivity may contribute to this effect. (Hypertension. 2000;35:685-690.)

Key Words: rabbits ■ cholesterol ■ vasoconstriction ■ cardiac output ■ total peripheral resistance

Excessive cholesterol alimentation causes hypercholesterolemia and eventually atherosclerosis.¹⁻³ Hypertension is another important risk factor for atherosclerosis.⁴ On the other hand, several studies have reported that blood pressure regulation, especially the control of vascular tone, is affected by high-cholesterol serum levels.³⁻⁵ For instance, vascular responsiveness to norepinephrine has been reported to be enhanced in animals maintained on a high-cholesterol diet.⁶⁻⁸ However, inconsistent results have been reported on vascular responsiveness to angiotensin II (Ang II). The constrictor response of aortic rings to Ang II was found to be markedly enhanced in one study³ and reduced in another study.⁹ With regard to other vascular beds, iliac artery rings from cholesterol-fed rabbits also did not exhibit an enhanced response to Ang II,⁹ and intrarenal Ang II infusion had a similar effect on renal vascular constriction in control rabbits and rabbits with a high-nutritional cholesterol intake.¹⁰ Because of these discrepant responses to Ang II in different isolated vascular beds, one can speculate that the altered cardiovascular control in the atherosclerotic rabbit model may not solely be confined to the vessel and end organ response. Despite the nonuniform responses to Ang II found in isolated vessels,³⁹ the effect of Ang II on arterial blood pressure was enhanced in atherosclerotic conscious rabbits.¹¹ Total peripheral resistance was not determined in the latter study; thus, it remains unclear whether this larger pressure increase relies on an enhanced vascular responsiveness. Alternatively, blood pressure controlling mechanisms, eg, the arterial baroreceptor reflex, could be altered in animals maintained on a high-cholesterol diet and, therefore, may be responsible for the larger pressure response to Ang II in intact animals. In particular, the vascular response to Ang II may be blunted to a lesser extent by the baroreceptor reflex in atherosclerotic animals. This would explain a stronger increase in total peripheral resistance in vivo, whereas the vascular response to Ang II would be unaltered in the absence of the baroreflex, ie, in isolated blood vessels. The present study was performed to examine the effect of systemically administered Ang II on arterial blood pressure and total peripheral resistance in the hypercholesterolemic rabbit. In addition, we determined arterial baroreceptor reflex sensitivity in rabbits maintained on a high-cholesterol diet to investigate whether this important blood pressure regulating mechanism is impaired by hypercholesterolemia and, therefore, can explain the different
responses to Ang II in isolated blood vessels and intact animals.

Methods

Experimental Animals and Atherogenic Diet

Nine-month-old locally bred rabbits of both genders (3.6±0.1 kg bw) were housed individually under constant climate conditions and had free access to drinking water. After an acclimatization period of 4 weeks, rabbits were randomly assigned to 1 of 2 groups: The control group received a standard diet of 130 g/d (n=12). The experimental group (n=11) was given the same basic diet, to which 2 g cholesterol and 20 mL sunflower seed oil were added. These diets were given for 12 weeks, before the high-cholesterol diet was discontinued and both groups received the same standard diet for an additional 6 weeks. Then, hemodynamic measurements were performed. Venous blood samples were drawn at the onset of the study, on 4 occasions during the high-cholesterol feeding period, and at the end of the study. Three days of noncholesterol feeding were allowed before blood sampling to avoid excessive lipid plasma levels and consecutive hemolysis. Serum values for total lipids, glucose, and the following enzymes were assessed: glutamic-oxalacetic-transaminase (GOT), glutamic-pyruvic-transaminase (GPT), lactate-dehydrogenase (LDH), and α-hydroxybutyrate-dehydrogenase (α-HBDH).

Animal Preparation and Experimental Protocol

Rabbits were anesthetized with urethane (1.5 g/kg bw, IV) and placed on a thermostat table (Zimmerman, Leipzig, Germany). Catheters for pressure measurements were positioned in the left ventricle, in the abdominal aorta, and in the vena cava. Additional catheters were implanted in both femoral veins for infusion of Ang II and injection of the indicator solution for determination of cardiac output. Pressure amplifiers provided the ventricular, arterial, and central venous pressure and the differentiated left ventricular pressure signal (LV dP/dt). Heart rate was obtained from the ECG, and end-diastolic pressure was obtained from the left ventricular pressure signal using the ECG as a trigger signal. The maximum of the LV dP/dt signal (LV dP/dtmax) and its ratio to the instantaneous pressure (LV dP/dtmax/P) were used as markers for myocardial contractility. Cardiac output was measured by the indocyanine dye dilution method, a modification of the technique described by Angell-James et al. A stabilization period of 30 to 45 minutes was allowed before hemodynamic measurements were performed in anesthetized and spontaneously breathing rabbits. An initial baseline period of 5 minutes was followed by an intravenous infusion of 0.4 µg·kg⁻¹·min⁻¹ Ang II. This dose was selected on the basis of both the literature and pilot experiments in which we applied doses in the range of 0.4 to 4.0 µg·kg⁻¹·min⁻¹. The dose of 0.4 µg·kg⁻¹·min⁻¹ is within the range of the upper plateau of the dose-response curve for blood pressure. Ang II infusion was stopped after 15 minutes, and the recording was continued for a final recovery period of 5 minutes. Cardiac output, stroke volume, and total peripheral resistance were determined in the initial baseline period and during the 3rd and 14th minute of Ang II infusion. Baroreceptor–heart rate reflex sensitivity was assessed by linear regressions between arterial pressure and heart period with the pressure increase and decrease that occurred in response to the onset of Ang II infusion (0.4 µg·kg⁻¹·min⁻¹) and its termination, respectively. These sections of the recordings lasted for ~3 minutes. Only linear regressions with correlation coefficients >0.85 were considered.

Eight hours after the hemodynamic measurements, both ventricles, the abdominal and thoracic portions of the aorta, the carotids, and the quadriceps muscle were taken for lipid chemical and histological examinations. The myocardium was inspected for necrotic lesions, scar tissue, and fibrosis. In addition, conduit vessels (aorta, carotids, and coronary arteries) and arterioles in the perivascular tissue of the aorta and carotids as well as within the myocardium were examined morphometrically with special attention to atherosclerosis.

Statistics

Data are mean±SEM. Comparisons between both dietary groups were performed by unpaired t tests. Time courses were tested by 2-way ANOVA (time versus dietary treatment). Post hoc t tests were performed to test individual differences, if the 2-way ANOVA revealed statistical significance. Statistical significance was assumed at P<0.05.

Results

Serum Lipids and Tissue Cholesterol

A high-cholesterol diet increased total serum lipid levels, which dramatically decreased 6 weeks after the high-cholesterol intake period, i.e., at week 18 (Figure 1A). Control values, however, were not fully restored. Serum glucose levels did not differ between dietary groups. The serum activities of GOT, GPT, LDH, and α-HBDH increased temporarily during the high-cholesterol feeding period and returned to normal at the end of the 6-week postcholesterol period.

Tissue cholesterol content in the aorta was increased 4-fold by high-cholesterol alimentation, whereas cholesterol content in the myocardium and skeletal muscle did not rise significantly (Figure 1B). The pathohistological effects of a high-cholesterol diet on the vascular system are shown in Figure 2. At week 18, a marked atherosclerosis was observed in the conduit vessels but not in the resistance vessels. The extent of the myocardial lesions (granulation tissue and scars) was only 1.3% on average. None of the animals included in this study had myocardial tissue damage of >4%. Fresh myocardial necroses were not observed.

Hemodynamic Effects of Ang II

To minimize potential influences of excessively high serum lipid and cholesterol levels on the hemodynamic measurements, these recordings were performed at week 18, i.e., 6
weeks after the high-cholesterol diet was terminated. Baseline hemodynamic conditions at week 18 are provided in Table 1. Significant differences were not found between the 2 groups. No hemodynamic signs of cardiac dysfunction were observed. Infusion of Ang II initially caused an increase in arterial blood pressure of ≈20 mm Hg in both groups. Despite the continuous infusion of Ang II, blood pressure gradually declined in both groups and reached baseline levels at the end of the infusion (minute 14) in the control group but not in the high cholesterol group (Figure 3, top). Ang II infusion also decreased heart rate in control rabbits (Figure 3, middle), which was probably caused by activation of the arterial baroreceptor reflex. In contrast, rabbits that had been maintained on a high-cholesterol diet for 12 weeks did not show a reduced heart rate to the same extent, indicating a reduced baroreceptor–heart rate reflex sensitivity. Left ventricular end-diastolic pressure increased significantly during Ang II infusion in both groups (Figure 3, bottom). The contractility index LV dP/dt\textsubscript{max}/P similarly decreased in both groups (Figure 3, bottom). Ang II–induced changes in cardiac output, mean arterial pressure, and total peripheral resistance at the 3rd and 14th minute of Ang II infusion are shown in Figure 4. A significantly diminished cardiac output was found for the group that was fed cholesterol. The increase in total peripheral resistance associated with the Ang II infusion was more pronounced in rabbits that received the atherogenic diet. At the end of Ang II infusion, this difference in the overall vascular response to Ang II reached statistical significance.

**Arterial Baroreflex Sensitivity**

The slope relating pulse interval to arterial blood pressure was less steep in the rabbits that received the high-cholesterol diet (Figure 5). This can be taken as a sign for a blunted baroreceptor–heart rate reflex sensitivity. This interpretation is further substantiated by the heart rate time course depicted in Figure 3. Although mean arterial pressure increased almost to a similar amount in both groups, or even slightly more in the high-cholesterol group at the end of the infusion, bradycardia was stronger in the control animals.
Discussion

This study demonstrates an enhanced increase in total peripheral resistance in response to systemically applied Ang II in atherosclerotic rabbits. In addition, a reduced baroreceptor reflex sensitivity was observed, giving rise to the hypothesis that the enhanced vascular responsiveness to Ang II in atherosclerosis is not solely related to an altered vascular function, but also relies on an impaired arterial baroreceptor reflex.

High-cholesterol feeding markedly increased total serum lipids and vascular cholesterol content (Figure 1) and thus caused atherosclerosis (Figure 2). Studies in isolated vessels from atherosclerotic rabbits are equivocal with regard to the vasoconstrictor response to Ang II. Dam and coworkers\(^9\) found a reduced response in aortic rings and no difference in

Baseline Hemodynamic Characteristics

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Standard Diet (n=12)</th>
<th>High-Cholesterol Diet (n=11)</th>
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<tr>
<td>SAP, mm Hg</td>
<td>113±4</td>
<td>120±7</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
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<td>DAP, mm Hg</td>
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<td>HR, bpm</td>
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<td>LV dP/dt(_{max}), mm Hg (\cdot ) s(^{-1})</td>
<td>6030±552</td>
<td>5779±780</td>
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<td>LV dP/dt(_{max})/P (s(^{-1}))</td>
<td>92±6</td>
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<td>CO, mL/min</td>
<td>731±100</td>
<td>769±80</td>
</tr>
<tr>
<td>SV, mL</td>
<td>2.5±0.3</td>
<td>2.8±0.4</td>
</tr>
<tr>
<td>TPR, mm Hg (\cdot ) L(^{-1}) (\cdot ) min</td>
<td>153±20</td>
<td>151±18</td>
</tr>
</tbody>
</table>

Baseline hemodynamic characteristics in rabbits fed a high-cholesterol diet and controls receiving a standard diet. Systolic, mean, and diastolic arterial pressure (SAP, MAP, DAP), heart rate (HR), central venous pressure (CVP), left ventricular end-diastolic pressure (LVEDP), the maximum of the slope of the left ventricular pressure curve and its ratio to the instantaneous pressure (LV dP/dt\(_{max}\), LV dP/dt\(_{max}\)/P), cardiac output (CO), stroke volume (SV), and total peripheral resistance (TPR) did not differ significantly.

Figure 3. Time course of mean arterial blood pressure (MAP), heart rate (HR), left ventricular end-diastolic pressure (LVEDP), and myocardial contractility index (LV dP/dt\(_{max}\)/P). ○: high-cholesterol diet, n=11. ●: standard diet, n=12. *P<0.05 absolute values during Ang II infusion vs baseline values.

Figure 4. Ang II–induced changes in cardiac output (CO), MAP, and total peripheral resistance (TPR). Gray bars: high-cholesterol diet, n=11. White bars: standard diet, n=12. *P<0.05 absolute values at the 3rd and 14th minute of Ang II infusion vs baseline values. #P<0.05 high-cholesterol diet vs standard diet.

Figure 5. Relationship between changes in MAP and interbeat interval (IBI) associated with initiation (positive MAP values) and termination (negative MAP values) of Ang II infusion. Solid line: high-cholesterol diet, n=11. Dashed line: standard diet, n=12. #:P<0.05 high-cholesterol diet (HCD) vs standard diet (SD).
iliac arteries. In contrast, Yang et al. found an enhanced response to Ang II in aortic rings. In vivo studies are also difficult to reconcile. For example, Hof and Hof describe an enhanced pressure effect of systemically administered Ang II, while Carroll et al. found that both the systemic pressure response and the renal vasoconstrictor response to intrarenal Ang II infusions were similar in control and atherosclerotic rabbits. This diversity within the literature prompted us to test whether the overall vascular responsiveness to Ang II is enhanced in atherosclerosis and whether an impairment of cardiovascular control mechanisms adds to this hyperresponsiveness. According to this hypothesis, the vasoconstrictor response to Ang II would be less effectively antagonized by the baroreflex and, consequently, cause a stronger increase in total peripheral resistance in atherosclerosis. This interpretation was, indeed, substantiated by the present study. First, total peripheral resistance increased more in response to systemically administered Ang II in atherosclerotic rabbits (Figure 4). Second, baroreflex sensitivity to the heart was markedly reduced in cholesterol-fed animals (Figure 5). Accordingly, the bradycardic response to the Ang II–induced hypertension was blunted in cholesterol-fed animals, which would be expected if the baroreflexes were impaired (Figure 3). Third, resistance vessels were excluded from the atherosclerotic process, suggesting a contribution of extravascular mechanisms to the increased vascular response to Ang II.

Recent in vitro experiments on the effects of Ang II in atherosclerotic vessels support our findings obtained in the whole animal: Yang and colleagues demonstrated a 5-fold increase in Ang II receptor expression, and Song et al. have observed an increased angiotensin-converting enzyme activity in atherosclerotic vessels. The latter group also found that Ang II type 1 receptor density increased in the medial lesion. Thus, a basis exists for assuming that the baroreflex blunted the vascular response to Ang II. Therefore, abnormal serum enzyme activities or excessively high serum levels of cholesterol and serum lipids were avoided. Thus, we cannot rule out that arterial blood pressure may have been higher during the 12 weeks of high-cholesterol feeding and returned to normotensive values within the postcholesterol period. Furthermore, the kidney, a key organ in the control of arterial blood pressure, may have effectively prevented hypertension in the present study. At least from a functional point of view, renal vascular resistance is not affected by a high-cholesterol diet nor is the renal vascular response to Ang II changed. When observed morphologically, the renal microvasculature seems to be protected, perhaps by an increase in cholesterol esterase.

A reduced afferent baroreceptor activity in atherosclerotic animals was first described by Angell-James. With an elegant approach, she performed nerve recordings from the aortic nerve while locally altering aortic arch pressure. Compared with controls, lower frequencies in the impulse discharges of afferent baroreceptor fibers in atherosclerotic rabbits were found, indicating that the afferent arch of the baroreflex is on fault in atherosclerosis. Like other investigators, we also found a reduced baroreceptor–heart rate reflex in atherosclerosis. Because primarily the afferent portion of the reflex is impaired in atherosclerosis, one can expect that the sensitivity of both the baroreceptor-sympathetic nerve activity reflex and the baroreceptor–heart rate reflex is reduced in atherosclerosis. Indeed, it has been demonstrated that baroreflex control of sympathetic renal nerve activity is depressed in conscious WHHL rabbits. Thus, the Ang II–induced increase in arterial blood pressure may elicit a smaller reduction in sympathetic nerve activity directed to the resistance vessels and, hence, may add to the enhanced vascular responsiveness to Ang II in vivo. There are several levels at which atherosclerosis can blunt the baroreflex. Li and associates have shown that endogenous oxygen-derived radicals, which can be produced by atherosclerotic vessels, attenuate the carotid nerve response to pressure changes. Furthermore, platelet activation in carotid sinuses, a common site of atherosclerotic lesions, which may facilitate platelet aggregation, markedly attenuates reflex-mediated changes in renal sympathetic nerve activity. Altered vessel wall structure as a result of hypercholesterolemia may also account for an attenuated baroreflex, because increased aortic and carotid vessel stiffness can change baroreflex characteristics. Yet, it seems unlikely that an attenuation of the arterial baroreflex is due to heart failure. Although interspersed scar tissue was detected in the ventricles, none of the hemodynamic characteristics showed any signs of a failing heart (Table 1, Figure 3). In addition, a reduced end-organ response to autonomic nervous discharge does not seem likely to account for the attenuated baroreflex sensitivity seen in this model of atherosclerosis. As mentioned above, isolated atherosclerotic vessels appear to be supersensitive to adrenergic transmitters.

In summary, this study provides data of Ang II effects on total peripheral resistance in atherosclerotic animals. Ang II increased total peripheral resistance by a greater degree in rabbits fed cholesterol. Furthermore, baroreceptor reflex sensitivity was markedly reduced. Thus, it is reasonable to assume that the baroreflex blunted the vascular response to Ang II to a lesser degree in atherosclerotic animals. In vivo, an attenuated sensitivity of the baroreceptor reflex may, therefore, add to the enhanced overall vascular responsiveness to Ang II associated with atherosclerosis.

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