Angiotensin Reactivity in the Cheek Pouch of the Renovascular Hypertensive Hamster

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SUMMARY Increased reactivity to vasoconstrictor agents and decreased arteriolar luminal diameter have been implicated in the maintenance of hypertension. The same hamster cheek pouch microvessels were tested for angiotensin I (Ang I) and angiotensin II (Ang II) reactivity before and 10 to 14 days after Grollman (two-kidney, one figure-8) or sham operation. Microvascular geometric parameters were measured before and after a maximal vasodilator dose of adenosine. Then maximal vasoconstrictions to Ang I or Ang II were measured: Ang I and Ang II were applied adjacent to arterioles (10^-2-10^-6 pmol) and venules (10^-7 pmol) in 10-μl aliquots for 1 minute. Blood pressure (178 ± 11/133 ± 8 mm Hg) of renovascular hypertensive hamsters was elevated significantly over blood pressure of sham-operated hamsters (120 ± 11/97 ± 10 mm Hg). No change was observed in venular geometry or reactivity in renovascular hypertensive hamsters. Arteriolar luminal diameter, wall thickness, wall/lumen ratio, and wall area were not altered in hypertensive hamsters in the normal or vasodilated state; vasodilator capacity was the same in all groups. Conversion of Ang I to Ang II (response to Ang I divided by response to Ang II) for first-order and third-order arterioles and third-order venules was 74 ± 5, 79 ± 3, and 72 ± 6%, respectively, and was unaltered in renovascular hypertensive hamsters. Although vessel geometry was not altered, there was a significant shift to the left of the Ang I and Ang II dose-response curves of first-order and third-order arterioles, indicating increased sensitivity to these vasoconstrictors. Increased microvascular responsiveness to Ang II may lead to an exacerbation of renovascular hypertension during periods of increased plasma renin activity. (Hypertension 12: 373-379, 1988)

KEY WORDS kininase II • renovascular hypertension • angiotensin II • adenosine • vasoactivity •

ELEVATED peripheral resistance in hypertension in humans and animal models has structural and functional components in the microvasculature. Previous work has demonstrated that, in the Grollman two-kidney, two figure-8 hypertensive hamster, increased reactivity of cheek pouch arterioles to the vasoconstrictor agonists norepinephrine and angiotensin II (Ang II) can occur without changes in arteriolar wall thickness and that the latter only occurs in later stages of hypertension. Furthermore, there is a greater dissipation of microvascular pressure in the hypertensive compared to the normotensive hamster despite the absence of changes in wall thickness.

Although in the rat the chronic phase of one-kidney, one figure-8 hypertension is characterized by normal plasma renin activity, either converting enzyme inhibition or Ang II antagonists lower blood pressure. These data can be explained by alterations in several components of the Ang II system: decreased catabolism of Ang II, increased generation of angiotensin I (Ang I) by vascular renin, increased vascular responsiveness to Ang II, or increased converting enzyme activity. Alternatively, captopril may result in increased activation of vasodilator mechanisms, as captopril can increase prostaglandin and bradykinin levels.

Using the two-kidney, one figure-8 hypertensive hamster as a model, we designed the present experiments to ask the following questions: 1) Is the cheek pouch microvasculature of this model hyperreactive to exogenous Ang II? 2) If so, is the hyperreactivity accompanied by structural modifications of microvessels? 3) Do cheek pouch microvessels of the hypertensive hamster have decreased reactivity to the vasodilator adenosine? 4) Is there increased ability to generate Ang II from exogenous Ang I in renovascular hypertension? In pursuit of these aims, the experiments were designed to allow
Materials and Methods

Microscopic Observation of the Microcirculation

The technique is a modification of that of Click et al. Female hamsters (Sasco, 6 weeks old, 140–160 g) were anesthetized with pentobarbital sodium (65 mg/kg body weight i.p.) and placed onto a temperature-controlled heating pad. The right femoral artery, for preoperative (prènormotensive or prehypertensive) experiments, or the left femoral artery, for postoperative (postnormotensive or posthypertensive) experiments, was cannulated for measurement of arterial blood pressure using a Statham P23Db (Gould, Instruments Division, Cleveland, OH, USA) transducer connected to a Gould Model 2400S recorder. The hair on the left side of the neck was removed carefully, thereby allowing transillumination from a fiberoptic bundle that was inserted into the cheek pouch under the baseplate. A 2-cm incision was made in the skin overlying the cheek pouch, and the skin was retracted so that the cheek pouch membrane was exposed. Small incisions were made over two pegs placed at opposite ends of the baseplate so that a suffusion chamber could be inserted over the baseplate. The chamber and baseplate were secured by a pursestring suture in the skin around the chamber. The baseplate holder then was removed, and the hamster was placed onto a temperature-controlled heating pad. The right femoral artery was ligated upstream, the cannula was removed, and the cheek pouch was sutured. The femoral artery was visible for the next 10 to 14 days, thereby allowing precise placement of peg holes for postsurgical microvascular observation. Then, the chamber and baseplate were removed and the skin over the cheek pouch was sutured. The femoral artery was ligated upstream, the cannula was removed, and the skin was sutured. This procedure allowed for measurement of the same arterioles and venules before and after Grollman or sham operation. Hamsters were not treated with antibiotics, and the cheek pouches had what appeared to be normal red blood cell flow, no venous stasis, and an overall normal appearance when tested for microvascular responsiveness after Grollman or sham operation.

Development of the Model

Hamsters that had been tested for microvascular reactivity were placed randomly into either a hypertensive group or a normotensive group. Renovascular hypertension was induced in 12 hamsters anesthetized with pentobarbital sodium (65 mg/kg i.p.) approximately 1 week after initial microvascular testing. The left kidney was exposed by a retroperitoneal approach and tied by a figure-8 ligature of 00 silk (Ethicon) after the method of Grollman. Ten sham-operated normotensive controls were handled similarly except there was no ligation of the kidneys. Postsurgical microvascular testing was performed 10 to 14 days later. The terms prènormotensive and postnormotensive refer to hamsters before and after sham operation, while prehypertensive and posthypertensive refer to hamsters before and after Grollman operation.
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Analysis of Data
Conversion of Ang I to Ang II was calculated by the following formula: \[ \text{percent conversion} = \left( \frac{\text{response to molar dose of Ang I}}{\text{response to molar dose of Ang II}} \right) \times 100. \] Inherent in this formula is the assumption that the cheek pouch does not respond to Ang I but requires prior conversion of Ang I to Ang II. This assumption was verified by preliminary experiments in which Ang I was found to be inactive in the presence of captopril. Since data presented as percentages generally are not distributed normally, data for percent conversion and percent vasodilation were transformed to their arc sine prior to subsequent statistical analysis.

Blood pressures, weights, and the microvascular parameters of luminal diameter, wall thickness, wall/lumen ratio, wall area, and conversion of Ang I to Ang II were compared by repeated-measures analysis of variance. Bonferroni comparisons were made for analyzing differences between treatment means. Dose-response curves for Ang II (arc sine of percent vasoconstriction vs log dose) were compared by analysis of covariance, and individual comparisons of slope or elevation were made by using a Newman-Keuls multiple range test. All data are expressed as means ± SEM, and significance was assigned to \( p \) values less than 0.05.

Results
Blood pressures and body weights of the hamsters used in this study are shown in Table 1. According to our criterion of hypertension (i.e., systolic blood pressure > 130 mm Hg), all hamsters that had the Grollman two-kidney, one figure-8 operation became hypertensive within 10 to 14 days. Both systolic and diastolic blood pressures were elevated significantly \( (p < 0.05) \) following induction of hypertension. In contrast to the elevated blood pressure seen in posthypertensive hamsters, blood pressures did not differ significantly between prenormotensive and postnormotensive hamsters.

After operation, the body weights were significantly depressed \( (p < 0.05) \) relative to preoperative weights (see Table 1). However, analysis of variance revealed no interaction between weight and treatment. The weights of posthypertensive hamsters were not significantly different from weights of postnormotensive hamsters. Thus, an effect on weight of hypertension per se was not evident.

Morphometric data from the microvessels are depicted in Table 2. Luminal diameter, wall thickness, wall/lumen ratio, and wall area were not altered in either posthypertensive hamsters or postnormotensive hamsters. After measurement of morphological parameters in vessels with tone, measurements were made in adenosine-dilated vessels (see Table 2). As observed in vessels with tone, luminal diameter, wall thickness, wall/lumen ratio, and wall area of vasodilated vessels were not altered in posthypertensive or postnormotensive hamsters.

Adenosine suffusion caused a vasodilation of small arterioles (3A). When vasodilator responses were compared before and after operation, it was found that the responses were decreased after operation relative to preoperative responses (Figure 1). However, this effect cannot be ascribed to hypertension since the decrease in vasodilator capacity in posthypertensive hamsters did not differ from that in postnormotensive hamsters. The effects of adenosine on 1A were small and variable. In prenormotensive hamsters, 1A dilated in the presence of adenosine and the magnitude of the dilation was unaltered by the operation. The 1A of prehypertensive and posthypertensive hamsters did not dilate in the presence of adenosine. The small vasodilation of 3V did not differ among the four groups.

Topical administration of Ang I and Ang II caused dose-dependent vasoconstrictions of 1A (Figures 2 and 3, top panels). When dose-response curves were generated in postnormotensive hamsters, responsiveness did not differ significantly from that in prenormotensive and prehypertensive hamsters. In contrast to the postnormotensive hamsters, there was increased microvascular responsiveness to Ang I and Ang II when tested on 1A in posthypertensive hamsters. This increase was manifested as a parallel shift to the left \( (p < 0.05) \) of the dose-response curve for Ang I in posthypertensive versus prehypertensive hamsters.

The vasoconstrictor responses of 3A to Ang I and Ang II are depicted in the bottom panels of Figures 2 and 3. The dose-dependent vasoconstrictions of 3A were not altered in postnormotensive hamsters. When dose-response curves were generated in posthypertensive hamsters, however, there was a parallel shift to the left of the Ang II dose-response curve.

| Table 1. Blood Pressures and Body Weights in Sham-Operated and Renovascular Hypertensive Hamsters |
|---|---|---|---|---|
| Variable | NT sham operation | HT 2K1F8 |
| | Preop (n = 10) | Postop (n = 10) | Preop (n = 12) | Postop (n = 12) |
| Systolic blood pressure (mm Hg) | 120 ± 4 | 120 ± 11 | 123 ± 5 | 178 ± 11* |
| Diastolic blood pressure (mm Hg) | 100 ± 6 | 97 ± 10 | 93 ± 5 | 133 ± 8* |
| Weight (g) | 148 ± 6 | 142 ± 3* | 152 ± 5 | 131 ± 4* |

Values are means ± SEM. NT = normotensive; HT = hypertensive; 2K1F8 = two-kidney, one figure-8.

*\( p < 0.05 \), compared with respective preoperative value.
Table 2. Geometric Characteristics of Microvessels in the Cheek Pouch of Normal and Hypertensive Hamsters

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-NT</th>
<th>Post-NT</th>
<th>Pre-HT</th>
<th>Post-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st-order arteriole</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L (µm)</td>
<td>104±9</td>
<td>106±6</td>
<td>99±5</td>
<td>100±5</td>
</tr>
<tr>
<td>W (µm)</td>
<td>15±1</td>
<td>14±1</td>
<td>12±1</td>
<td>12±1</td>
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<tr>
<td>W/L</td>
<td>0.15±0.02</td>
<td>0.13±0.01</td>
<td>0.13±0.01</td>
<td>0.13±0.01</td>
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<tr>
<td>A_w (µm²)</td>
<td>5600±660</td>
<td>5200±360</td>
<td>4400±520</td>
<td>4400±350</td>
</tr>
<tr>
<td>3rd-order arteriole</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L (µm)</td>
<td>23±1</td>
<td>24±1</td>
<td>26±1</td>
<td>24±1</td>
</tr>
<tr>
<td>W (µm)</td>
<td>5±1</td>
<td>6±1</td>
<td>6±1</td>
<td>7±1</td>
</tr>
<tr>
<td>W/L</td>
<td>0.22±0.02</td>
<td>0.24±0.02</td>
<td>0.25±0.03</td>
<td>0.28±0.03</td>
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<tr>
<td>A_w (µm²)</td>
<td>440±62</td>
<td>510±61</td>
<td>640±65</td>
<td>660±100</td>
</tr>
<tr>
<td>3rd-order venule</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L (µm)</td>
<td>39±2</td>
<td>42±3</td>
<td>41±2</td>
<td>38±1</td>
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<tr>
<td>W (µm)</td>
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<td>4±1</td>
<td>5±1</td>
<td>5±1</td>
</tr>
<tr>
<td>W/L</td>
<td>0.12±0.01</td>
<td>0.10±0.01</td>
<td>0.14±0.01</td>
<td>0.13±0.01</td>
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<tr>
<td>A_w (µm²)</td>
<td>650±92</td>
<td>630±74</td>
<td>780±67</td>
<td>700±74</td>
</tr>
</tbody>
</table>

Values are means ± SEM. NT = normotensive; HT = hypertensive; L = luminal diameter; W = wall thickness; W/L = wall/lumen ratio; A_w = wall area.

Responses in arterioles were more responsive to Ang II than were venules. The maximal vasoconstrictor response of 3V was 20%. Furthermore, the vasoconstrictor response to 0.1 pmol was greater than that to 0.01 pmol, it did not differ from that to 1.0 pmol. In contrast to the responses in arterioles, responses in venules did not differ significantly among prenormotensive and postnormotensive or prehypertensive and posthypertensive hamsters.

Microvascular conversion of Ang I to Ang II was quantified by comparison of vasoconstrictor responses to equimolar amounts of Ang I and Ang II. Conversion was calculated at two doses of Ang I and Ang II (0.01 and 0.1 pmol) for both orders of arterioles and one dose of Ang I and Ang II (0.1 pmol) for venules. Since the calculated conversions were not dose-dependent in the arterioles, the conversions at the two doses were averaged for each hamster before statistical analysis. As seen in Figure 4, conversion averaged approximately 70% in microvessels of the cheek pouch and did not differ among the types of microvessels. Conversion of Ang I to Ang II did not differ among prenormotensive and postnormotensive or prehypertensive and posthypertensive hamsters.
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Figure 3. Vasoconstrictor responses to local administration of Ang II in first-order (1A; top panel) and third-order arterioles (3A; bottom panel). Drug was applied in 10-μL aliquots over a period of 1 minute. Values on the abscissa represent the total amount of Ang II applied over the 1-minute period. The log dose-response curves for posthypertensive hamsters were shifted significantly to the left of those of prehypertensive, prenormotensive, and postnormotensive hamsters (p < 0.05) for both 1A and 3A.

Discussion

The two-kidney, one figure-8 Grollman operation, similar to the two-kidney, two figure-8 operation, produces hypertension in a reproducible manner.1-3, 16-18 In the present study, morphological parameters and angiotensin reactivity were measured in selected 1A, 3A, and 3V of the hamster cheek pouch before and after induction of renovascular hypertension. Our data indicate that 10 to 14 days after induction of hypertension, reactivity of 1A and 3A is elevated in the absence of any changes in luminal diameter, wall thickness, wall/lumen ratio, or wall area during either the resting or vasodilated state. Vasodilator capacity was not altered in hypertension. Microvascular conversion of Ang I to Ang II was comparable in arterioles and venules and was not altered in hypertension. If these data can be extrapolated to other vascular beds, they indicate that, in this model, hypertension is not caused by increased peripheral resistance resulting from luminal encroachment and that the increased reactivity is not secondary to an increased media.

To our knowledge, this is the first study in which geometric and physiological parameters were obtained from the same microvessels before and after induction of hypertension. As in other microvascular beds, a given vessel in the hamster cheek pouch can give rise to daughter vessels that vary greatly in luminal diameter. Thus, to compare vessels of a given order, rigid criteria must be set for defining when a given branch constitutes a higher order vessel; measurements such as daughter/parent ratios of luminal diameter or wall parameters are convenient criteria. However, such an indirect technique is subject to more error than the technique used in the present study.

The parallel shift to the left of the angiotensin dose-response curve in renovascular hypertension indicated an increased sensitivity. This interpretation is supported in that wall thickness, wall/lumen ratio, and wall area were not changed in any order of vessel studied. Also, since there were no changes in luminal diameter and wall parameters, the potential role for alterations in static vascular geometry in the maintenance of high blood pressure 10 to 14 days after induction would be minimal. Similarly, previous studies in the two-kidney, two figure-8 hamster revealed that after 4,1 8,1 and 141, 2 days of hypertension, diameters and wall/lumen ratios of similarly sized arterioles (40-54 μm) were unchanged. Nevertheless, vasoconstrictor responsiveness to Ang II was increased.1 Although only one dose of Ang II was used, thereby precluding direct comparison with our data, sensitivity to norepinephrine also was increased.1 Indeed, although in two-kidney, two figure-8 hypertension diameters of 1A are increased and diameters of 3V are decreased whereas in two-kidney, one figure-8 hypertension they are unchanged,1 the models are similar in that a given branching order of arteriole can be hyperresponsive in the absence of concomitant changes in structural parameters.

The increased responsiveness to Ang II may be a consequence of increased arteriolar pressure, as pressures are elevated in models of renovascular hypertension in hamsters.3 Gore19 has demonstrated that in some arteriolar segments wall stress is normally less than the stress at which arterioles...
maximally respond to norepinephrine. Inasmuch as wall stress is directly related to transmural pressure, hypertension may increase the stress toward the more optimal value. Thus, the increased responsiveness to Ang II may be a function of the physical properties of arteriolar smooth muscle rather than receptor or postreceptor properties.

Increased sensitivity to Ang II in hypertensive hamsters could result in decreased static measurements of basal arteriolar diameter. Several factors could contribute to the presence of normal diameters, such as decreased levels of Ang II (e.g., decreased synthesis secondary to decreased plasma or vascular renin reactivity or increased catabolism of Ang II) or increased activation of vasodilator mechanisms. Although the hamster cheek pouch is tachyphylactic to Ang II, it is unlikely that increased tachyphylaxis in the hypertensive hamsters could explain our data, as increased tachyphylaxis should result in a decrease in sensitivity to exogenous Ang II. It is also unlikely that angiotensinase activity is increased in the hypertensive hamsters, as this should also result in decreased sensitivity to exogenous Ang II. Whether Ang II levels are decreased or whether there is activation of vasodilator mechanisms cannot be determined from our data. Whatever the mechanism for the measurements of normal diameter in the static phase in the presence of increased sensitivity to Ang II, the data do not support a role for the renin-angiotensin system or reduced lumen diameters in the maintenance of high blood pressure 10 to 14 days after induction. The elevated blood pressure probably is secondary to increased plasma volume resulting from decreased kidney function. In the two-kidney, two figure-8 hypertensive hamsters, plasma volume is elevated 10 to 16 days after induction of hypertension.20 Alternative mechanisms for maintenance of elevated blood pressure in our model are functional or anatomical rarefaction,21-24 increased peripheral resistance proximal to the cheek pouch,25 dynamic alterations in vasomotor activity, and, possibly, increased sympathetic tone, which supports the increase in blood pressure but does not reduce the diameter of the cheek pouch arterioles because they are not innervated.

Converting enzyme (kininase II) activity has been demonstrated in the perfused canine hindleg26 and mesentery27 among other vasculatures. Within the vasculature, converting enzyme activity resides predominantly in the endothelium.28 It has been reported that cultures of endothelial cells from human pulmonary arteries convert three to five times as much Ang I to Ang II as do endothelial cells from human pulmonary veins.30 In the hamster cheek pouch, the present data and that of Shepard et al.31 demonstrate equal conversion in arterioles and venules. The reasons for this discrepancy between in vivo data in the hamster cheek pouch and the in vitro human data are unclear. Converting enzyme of cultured endothelial cells from veins may be more labile than that in arterial endothelial cells. In support of this concept is the finding of Ryan et al.28 that, within the perfused lung, endothelial cells of capillaries and venules have more converting enzyme than do endothelial cells of arteries. It is also conceivable that endothelial cells from microvessels differ from those from large blood vessels. Alternatively, our apparent equality of conversion between arterioles and venules may be a reflection of increased diffusion of the Ang I to the venular endothelium. We think this is unlikely since 1A and 3A, with greatly different wall thicknesses, had comparable conversion. Presumably, the lack of a difference between arteriolar and venular production was due to a high enzyme/substrate ratio. In the two-kidney, one figure-8 hypertensive hamster, converting enzyme activity is normal. Thus, this model is like the two-kidney, two clip and one- kidney, one clip rat22 but is unlike the two-kidney, one clip rat, in which converting enzyme activity is elevated.32Whether increased converting enzyme activity plays a role in the maintenance of an elevated blood pressure is thus dependent on the model of renovascular hypertension.

In summary, an increased microvascular sensitivity to Ang II in cheek pouch vessels of the renovascular hypertensive hamster was demonstrated. This change in sensitivity occurred in the absence of changes in conversion of Ang I to Ang II or of changes in vessel geometry. This study also showed that use of the hamster cheek pouch provides the opportunity to quantitate morphological and physiological parameters of given microvessels before and after an experimental manipulation. This technique holds promise for study of a number of experimental models of pathological states, such as hypertension and diabetes. Furthermore, this technique has potential in experiments from a number of protocols in which physiological parameters are correlated with morphological parameters obtained from light or electron microscopy. Careful placement of dye adjacent to areas from which physiological data are obtained would facilitate subsequent location during preparation of the tissue for microscopy.

References

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