Increased Vascular Sensitivity to Angiotensin II in Psychosocial Hypertensive Mice

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SUMMARY CBA mice develop hypertension when placed in complex population cages that facilitate social interactions and competition for territory. After 1 month, these mice have normal plasma renin levels, but blockade of converting enzyme lowers blood pressure to normal. To test the possibility that this normal-renin hypertension is caused by enhanced pressor responsiveness to angiotensin II (All), we examined the effects of All on hindquarter and renal vasculatures from 13 hypertensive and 13 normotensive mice. Both vascular beds were pump-perfused at a constant flow with plasma substitute. Optimal perfusion flows and basal pressures were similar in hindquarter (8 ml/100 g/min; 60 mm Hg) and renal vasculatures (130 ml/100 g/min; 50 mm Hg) from normotensive and hypertensive mice. Threshold constrictor responses to All were elicited at a significantly lower dose in both vasculatures of hypertensive mice than in those of normotensive mice. Maximal pressor responses to All were greater in the hindquarters of hypertensive mice than in those of normotensive mice, but were not different in the renal vasculatures of the two groups. Vasoconstrictor sensitivity to norepinephrine was also increased in the hindquarters of hypertensive mice; however, the changes in threshold and maximal pressor response were less than for All. Responsiveness to norepinephrine in the renal vasculatures of hypertensive mice was not different from that in the kidneys of normotensive mice. We conclude that the hyperresponsiveness to All in the resistance vessels plays an important role in maintaining elevated blood pressure in this psychosocial model of hypertension.

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KEY WORDS • hindquarter vasculature • renal vasculature • norepinephrine

CBA Agouti mice develop sustained hypertension when placed in complex population cages that facilitate social interactions and competition for territory.1-3 During the first 2 days of social interaction, plasma renin activity (PRA) in these mice is greatly elevated.2,3 However, after 3 to 4 weeks, PRA declines to values exhibited by normotensive mice housed in a traditional manner. During later stages of social interaction (10 to 11 months), PRA rises in hypertensive mice in association with the development of renal disease. During the prolonged middle period of normal-renin hypertension (2 to 9 months), there is no correlation between PRA and the magnitude of pressure elevation, and yet blockade of converting enzyme abolishes the hypertension. These observations suggest the possibility that pressor responsiveness to angiotensin II (All) is enhanced during the normal-renin phase of psychosocial hypertension. The goal of the present study was to test this hypothesis.

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Methods

The strain of CBA Agouti mice used in these experiments was bred in the University of Southern California Laboratories; the details of husbandry have been described previously.1 Briefly, the young were weaned at 18 to 21 days and then placed individually into 0.5 liter glass jars. At 4 months, these "isolates" were placed into special population cages along with an equal number of non-isolated females and males of the same age; these cages consist of six standard boxes formed into a circle by narrow connecting tubes with a central feeding and watering place connected to each box by radial spokes. The males, under these conditions, are highly aggressive, fail to establish a social hierarchy, and develop hypertension within the first week. Male mice raised in a traditional laboratory manner served as controls. Systolic blood pressures were measured in the conscious state by a tail cuff technique, as described previously.4 All mice were fed a standard commercial diet (Purina).

Studies were performed on age-matched hypertensive (2 months of social interaction) and normotensive mice in the Department of Physiology, University of Michigan. Experiments were completed within 7 days after the removal of the hypertensive mice from the population cage. Previous studies1 have shown that the hypertension is not altered during this 1-week interval. The mice were anesthetized with sodium pentobarbital...
(Nembutal, 35–50 mg/kg, i.p.) and a midline laparotomy performed. In 16 mice, the hindquarters were perfused through a catheter placed in the abdominal aorta proximal to the iliac bifurcation. In 10 mice, the left kidney was perfused through a catheter placed distally into the abdominal aorta and advanced to the origin of the left renal artery; the aorta was then ligated proximal to the left renal artery. In both preparations, the vena cava was opened to facilitate free exit of perfusate. The mouse was terminated by pneumothorax, and the renal or hindquarter vasculature was perfused in situ at a constant flow using a Harvard perfusion pump (Model 1202). Perfusion flows in both preparations were adjusted until perfusion pressure was approximately 50 mm Hg. Preliminary experiments indicated that pressor responsiveness to norepinephrine (5 μg) was maximal in both preparations at these flow rates (hindquarter = 8 ml/100 g/min; renal = 130 ml/100 g/min). The perfusate was maintained at 37°C and saturated with 95% O₂, 5% CO₂. The pH of the solution was 7.4 and the composition (mmol/liter) was as follows: NaCl, 130; KCl, 4.7; KH₂PO₄, 1.18; MgSO₄·7H₂O, 1.17; CaCl₂·2H₂O, 1.6; NaHCO₃, 14.9; dextrose, 5.5; CaNaEDTA, 0.03; and Ficoll 70 (Pharmacia AB), 0.71. Perfusion pressure was monitored with a Statham pressure transducer and a Grass Model 7 polygraph. Following isolation of renal or hindquarter vasculatures, an equilibration period of 30 minutes was allowed before the experimental protocol was begun.

Drugs used were norepinephrine (Levophed bitartrate, Breon Laboratories, New York, New York), angiotensin II (Sigma Chemical Company, St. Louis, Missouri), and papaverine hydrochloride (Eli Lily & Company, Indianapolis, Indiana). Doses of drugs were delivered in a constant volume (50 μl) through an injection port proximal to the perfusion pump. A 2 to 3-minute dosing cycle was used. Following drug administration, perfusate flow rates were increased and decreased to determine pressure-flow relationships.

All values presented in the text and in the figures are means ± standard error of the mean (SEM). Student's t test was used to determine statistical differences between experimental groups. Individual threshold (ED₅₀) and ED₉₀ values were computed by logit transformation. A p value less than 0.05 was considered to be statistically significant.

**Results**

At the time of experimentation, the systolic blood pressure of the psychosocial hypertensive mice was significantly higher than that of control normotensive mice (156 ± 3 vs 122 ± 2 mm Hg respectively; p < 0.05). Body weights of mice in the two groups were comparable (psychosocial hypertensive = 31 to 34 g; normotensive = 32 to 37 g).

At the flow rates used in these experiments, both the renal and hindquarter vasculatures were maximally dilated; bolus injections of papaverine into the vascular beds had no effect on perfusion pressure, indicating atonic preparations. Perfusion pressure in both preparations remained stable for the duration of the experiment (2 to 3 hours). Figure 1 shows the contribution of the hindquarter (left panel) and renal vasculature (right panel) to perfusion pressure at three rates of perfusate flow. At all flow rates, the average vascular pressures were comparable in respective preparations from hypertensive and normotensive mice. At the end of the experiments, perfused kidneys weighed 106% ± 3% of the nonperfused, contralateral kidney weight, indicating minimal edema formation. Perfused kidneys from psychosocial hypertensive mice (0.35 ± 0.01 g;
n = 5) were similar in weight to those from normotensive mice (0.34 ± 0.01 g; n = 5). Perfused hindquarters weighed 110% ± 5% of the nonperfused hindquarters (isolated from mice that had kidney perfusions); perfused hindquarters from hypertensive mice (10.1 ± 0.6 g; n = 8) were similar in weight to those from normotensive mice (9.9 ± 0.9 g; n = 8).

Bolus injections of All (0.0005 — 50 µg, fig. 2) or norepinephrine (0.0005 — 50 µg, fig. 3) increased perfusion pressure in both preparations from hypertensive and normotensive mice. Vascular responses to All and norepinephrine in hindquarter preparations from hypertensive mice (left panels, figs. 2 and 3) were greater than those in the hindquarters from normotensive mice. In kidneys from hypertensive mice, vascular responses to low doses (0.0005 — 0.05 µg) of All were augmented compared to those in kidneys from normotensive mice, whereas high doses of the drug (0.5 — 50 µg) produced similar changes in perfusion pressure in the two groups (right panel, fig. 2). Pressor responsiveness to norepinephrine in kidneys from hypertensive mice was similar to that in kidneys from normotensive mice at all doses of the drug (right panel, fig. 3).
To interpret the results in terms of sensitivity to the dose of agonist, pressor response for each vascular preparation was normalized to its maximal response, and the doses of the agonist producing 10% (threshold; ED_{10}) and 50% maximal responses (ED_{50}) were determined (table 1). Threshold and ED_{50} values for All and norepinephrine were lower in hindquarter preparations from hypertensive mice than in those from normotensive mice, indicating increased vascular sensitivity to the agonists. In the renal vasculature of hypertensive mice, threshold and ED_{50} values for All were significantly lower than those in the renal vasculature of normotensive mice. Threshold and ED_{50} values for norepinephrine in the renal vasculature of hypertensive mice were not different from those in the renal vasculature of normotensive controls.

The magnitude of the leftward shift in ED_{50} values for All in hindquarter and renal preparations from hypertensive mice exceeded the shift in ED_{50} for norepinephrine. To quantitate this relationship, a "shift factor" was calculated that expressed the magnitude of the leftward shifts in the dose-response curves relative to the mean ED_{50} values of the normotensive preparation, according to the following equation:

\[
\text{shift factor} = \frac{-\log \text{ED}_{50} \text{ normotensive} - (-\log \text{ED}_{50} \text{ hypertensive})}{-\log \text{ED}_{50} \text{ normotensive}}
\]

The shift factors for All were significantly greater in vascular preparations from hypertensive mice [renal = 0.59 ± 0.10 (n = 5); hindquarter = 0.30 ± 0.07 (n = 8)] than those for norepinephrine [renal = 0.16 ± 0.10 (n = 15); hindquarter = 0.10 ± 0.04 (n = 8); p < 0.05].

### Discussion

This study demonstrates that vascular sensitivity to All is increased in renal and hindquarter vascular beds in CBA mice after 2 months of psychosocially induced hypertension. This change in sensitivity to the peptide was greater in the renal vasculature than in the hindquarter vasculature, as evidenced by the magnitude of the shift to the left in the dose-response curve relative to respective normotensive preparations. Sensitivity to norepinephrine was also increased in the hindquarter vasculature of hypertensive mice, whereas responsiveness to the catecholamine was not significantly altered in the renal vascular bed of these animals. The change in sensitivity to norepinephrine in hindquarters of hypertensive mice was less than that for All, based on the magnitude of the leftward shifts in the dose-response curves relative to respective curves in normotensive preparations.

One possible explanation for the altered responsiveness of the vasculature in psychosocial hypertensive mice is that the resistance vessels had undergone a structural adaptation involving media hypertrophy accompanied by narrowing of the vessel lumen. Such a structural alteration would result in a greater change in flow resistance when the smooth muscle contracted in response to vasoactive stimuli. However, based on the similarity in pressure-flow relationships of hypertensive and normotensive vascular beds, it is doubtful that a significant lumen narrowing occurred in these hypertensive mice. If an increased media thickness without lumen narrowing were a major factor, the dose-response relationships to both norepinephrine and All should have been affected to the same extent. This was obviously not the case in either vascular preparation examined. In both preparations from hypertensive mice, the magnitude of the shift in the dose-response curves depended upon the agonist used (All > norepinephrine). Furthermore, the change in sensitivity to All in the hindquarter vasculature was accompanied by an increased maximal response to the peptide, whereas that in the renal vasculature was not.

It is likely that functional alterations in the vascular smooth muscle contributed to the increased responsiveness to All and norepinephrine in hypertensive mice. This study does not identify the precise mechanisms responsible for these alterations. It may be that receptor number and/or affinity for norepinephrine and All is altered in the vasculature of hypertensive mice. Alternatively, receptor activation might lead to a greater smooth muscle response due to an altered excitation-contraction coupling. It is also possible that reduced degradative mechanisms for norepinephrine (neuronal

### Table 1. Threshold (ED_{10}) and ED_{50} Values

<table>
<thead>
<tr>
<th>Vascular preparation</th>
<th>ED_{10} (ng)</th>
<th>ED_{50} (ng)</th>
<th>ED_{10} (ng)</th>
<th>ED_{50} (ng)</th>
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</thead>
<tbody>
<tr>
<td><strong>Hindquarter vasculature</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Hypertensive mice (n = 8)</td>
<td>26 ± 10*</td>
<td>219 ± 106*</td>
<td>33 ± 10*</td>
<td>580 ± 116*</td>
</tr>
<tr>
<td>Normotensive mice (n = 8)</td>
<td>128 ± 38</td>
<td>657 ± 157</td>
<td>104 ± 22</td>
<td>1,005 ± 208</td>
</tr>
<tr>
<td><strong>Renal vasculature</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertensive mice (n = 5)</td>
<td>0.5 ± 0.1*</td>
<td>5.3 ± 1.9*</td>
<td>8.2 ± 1.9</td>
<td>65.8 ± 24.6</td>
</tr>
<tr>
<td>Normotensive mice (n = 5)</td>
<td>12.3 ± 10.7</td>
<td>87.1 ± 67.3</td>
<td>11.1 ± 1.8</td>
<td>99.4 ± 24.4</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Individual ED_{10} and ED_{50} values were computed by logit transformation.

*Statistically significant difference between hypertensive and normotensive mice.
uptake, extra-neuronal uptake, etc.) and All (angiotensinases) may partially explain the increased sensitivity to each agonist. This study does demonstrate that the functional changes are not specific to the anatomical location of the vascular bed.

The hemodynamic importance of increased sensitivity to All and norepinephrine depends on receptor interaction with endogenous levels of the agents and on other humoral and neurogenic modulators of vascular tone. With respect to All, this may be particularly important, since previous studies have shown that PRA (and, presumably, plasma All concentration) is normal at the stage of psychosocial hypertension examined in this study; yet blockade of converting enzyme activity lowers blood pressure in these mice. Increased vascular sensitivity to All could explain how normal levels of the peptide could cause the hypertension.

The vascular changes that characterize psychosocial hypertension in mice closely resemble those reported for two-kidney, one clip (2K1C), renal hypertension in rats. Spontaneously hypertensive rats and mineralocorticoid hypertensive rats differ from normotensive controls in that vascular sensitivity to norepinephrine is increased in the renal vasculature. Thus, the vascular changes in psychosocial hypertensive mice may be related to the early elevation in PRA, which also characterizes 2K1C, renal hypertension in rats.

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