Effect of Age and Spontaneous Hypertension on the Tachyphylaxis to 5-Hydroxytryptamine and Angiotensin II in the Isolated Rat Kidney

CHRISTIAN DE MEY, M.D., AND PAUL M. VANHOUTTE, M.D.

SUMMARY The isolated and perfused kidney of the mature spontaneously hypertensive rat (SHR) exhibits an increased vascular reactivity and a delayed tachyphylaxis to 5-hydroxytryptamine, when compared to weight-matched normotensive animals. To evaluate the influence of the duration of the hypertensive state on these differences, the vascular reactivity to 5-hydroxytryptamine was determined in isolated kidneys from age-matched normotensive and spontaneously hypertensive rats of 3.5, 6, and 12 months of age. Responses to increasing doses of 5-hydroxytryptamine were compared. At all ages the responses to the agonist were greater in the SHR than in the control rats. In the normotensive rats, the sensitivity to the monoamine decreased, while the maximal response increased with aging. The vascular reactivity to increasing doses of 5-hydroxytryptamine was not altered by aging in the SHR. There was a significant correlation between the maximal vasoconstrictor response to 5-hydroxytryptamine in the isolated kidneys and the systolic arterial blood pressure (SBP) of the donor rats. Maximal constrictor responses to 5-hydroxytryptamine were repeated at given intervals. Tachyphylaxis to 5-hydroxytryptamine was decreased in hypertensive rats compared with normotensive rats at 3.5, 6, and 6 months of age. Tachyphylaxis to 5-hydroxytryptamine was not affected by either age or hypertension. There was no cross-tachyphylaxis between 5-hydroxytryptamine and angiotensin II (AI). Lowering the Ca²⁺ concentration of the perfusate did not affect tachyphylaxis to either 5-hydroxytryptamine or AI. The present experiments indicate that the delayed tachyphylaxis to 5-hydroxytryptamine in the kidneys of SHR is due to a specific alteration of the vascular smooth muscle cells, which may be the consequence of premature aging.

KEY WORDS • tachyphylaxis • age • 5-hydroxytryptamine • angiotensin II • kidney • hypertension duration • vasoconstriction • smooth muscle

THE ability of the renal blood vessels to constrict in response to 5-hydroxytryptamine is increased in mature spontaneously hypertensive rats (SHR).¹ Tachyphylaxis to repeated exposure of the renal blood vessels to maximal doses of 5-hydroxytryptamine is significantly decreased in mature SHR when compared with normotensive Kyoto Wistar and Wistar rats of the same sex and weight.¹ The delayed tachyphylaxis to 5-hydroxytryptamine in SHR is not due to interference with adrenergic mechanisms and does not depend on endogenous 5-hydroxytryptamine.³ The present experiments were performed to evaluate the effect of prolonged exposure to an increase in arterial blood pressure (BP) on the reactivity of the kidney vessels and the rate of development of tachyphylaxis to 5-hydroxytryptamine, and to compare the latter with that occurring on repeated administration of angiotensin II (AI).

METHODS

Male and female spontaneously hypertensive rats of the Okamoto-Aoki strain (SHR), and normotensive Wistar-Kyoto control rats (WKY), and inbred Wistar rats (WIS) were used in this study. Animals were matched for sex and age (3-4, 5, 6, 12, and 13-14 months old).

Rats were anesthetized (pentobarbital-sodium, 50 mg/kg, i.p.), and the left renal artery was cannulated via the aorta. The kidney was perfused at constant flow by means of a roller pump (Gilson, Minipuls II), with Tyrode solution of the following composition (mM): NaCl, 137; KCl, 2.7; CaCl₂, 1.8; MgCl₂, 1.1; NaHCO₃, 12.0; NaH₂PO₄, 0.42; D(+)-glucose, 5.6; aerated with CO₂ 5% in O₂. In certain experiments, the
CaCl₂ concentration of the Tyrode solution was decreased to $1.8 \times 10^{-4}$ mM, in equimolar replacement with NaCl. The kidney was then isolated by cutting the aorta, renal vein, and ureter, and was placed in a chamber at 37°C. Renal vascular constrictor responses were recorded as increases in perfusion pressure, downstream from the pump while the flow was maintained constant at the optimal level; the optimal flow rate and the perfusion pressure at that flow rate were not different in kidneys from age-matched WKY and SHR. Drugs (5-hydroxytryptamine or All) were administered by bolus injection of constant volumes (0.02 ml) into the perfusion system close to the kidney. A minimum dosing cycle of 3 minutes was used; this time period was extended when necessary to obtain complete return to baseline perfusion pressure.

The sensitivity of the vascular smooth muscle cell to 5-hydroxytryptamine was evaluated by determining the dose that evoked 20% of the maximal response (ED₂₀), and the structural changes by alterations of the maximal response. The rate and degree of tachyphylaxis to repeated administration of $2 \times 10^{-4} g$ 5-hydroxytryptamine or of $2 \times 10^{-5} g$ All were evaluated as the progressive decrease of the amplitude of the vascular responses expressed as percent of the response to the first dose; earlier work has shown that these concentrations of 5-hydroxytryptamine and All evoke maximal responses in the perfused rat kidney.

The experiments were conducted in parallel on preparations from normotensive and hypertensive rats. In the 12-month age group, the normotensive controls consisted of 50% WKY and 50% WIS rats; no significant differences were observed between the two strains. In the other age groups, only WKY served as normotensive controls. Except when otherwise stated, each experimental group consisted of six kidneys taken from different rats. Student's t test for unpaired observations was used throughout the study to evaluate differences between means; p values smaller than 0.05 were considered to be statistically significant. Only significant differences will be discussed.

**Results**

**Vascular Reactivity to 5-Hydroxytryptamine**

Responses to increasing doses of 5-hydroxytryptamine were larger in the isolated perfused kidney of SHR of all age groups compared with those of age-matched normotensive controls (fig. 1). The ED₂₀ and ED₅₀ were lower in kidneys of 6- and 12-month-old SHR compared with age-matched normotensive rats (table 1). The ED₂₀ and ED₅₀ were not altered by age in hypertensive animals from 3.5 up to 12 months of age. In normotensive rats, the sensitivity to 5-hydroxytryptamine was higher in the youngest group. The amplitude of the maximal response to 5-hydroxytryptamine (table 1) was not influenced by aging in hypertensive rats from 3.5 up to 12 months of age and was not correlated with systolic blood pressure (SBP). In normotensive rats, the amplitude of the maximal response increased with aging and was
correlated with SBP. The pooling of normotensive and hypertensive rats yielded a significant correlation between maximal responses to 5-hydroxytryptamine and SBP (fig. 2).

**Tachyphylaxis to Repeated Dosing of 5-Hydroxytryptamine**

The amplitude and duration of the vascular responses to repeated administration of $2 \times 10^{-8}$ g 5-hydroxytryptamine, with a dosing cycle of 6 minutes, progressively decreased in both normotensive and hypertensive rats. At 3.5, and 6, but not at 12, months of age, the tachyphylaxis to 5-hydroxytryptamine was less pronounced in kidneys from SHR than in those of age-matched control animals. The depression of the vascular response with repeated administration of high doses of 5-hydroxytryptamine was lower in the eldest group of both normotensive and hypertensive rats compared with younger animals (fig. 3).

**Figure 2.** Correlation of the vascular response (increase in perfusion pressure, mm Hg) to a high dose of 5-hydroxytryptamine ($2 \times 10^{-8}$ g) in the isolated perfused kidney of spontaneously hypertensive and normotensive rats, with the indirectly measured systolic blood pressure (mm Hg). For pooled group of normotensive rats (---): $y = a_1 x + a_0$; $a_1 = 0.37; a_0 = 70; r^2 = 0.69$. For pooled SHR, $a_1 = 11; a_0 = 159; r^2 = 0.16$. For pooled SHR and normotensive rats (---): $a_1 = 0.50; a_0 = 56.2; r^2 = 0.71$.

**Figure 3.** Progressive depression of the vascular responses of the isolated perfused kidney of hypertensive rats (SHR) and normotensive rats (WKY) with repeated administration of $2 \times 10^{-8}$ g 5-hydroxytryptamine. Dosing cycle = 6 minutes. Vascular responses are expressed as % of the amplitude of the first response and shown as means ± SEM. * = significantly different from normotensive rats of the same age group: $p < 0.05$. 
Tachyphylaxis to Repeated Dosing of Angiotensin II

Angiotensin II (2 × 10^-9 g) caused comparable vasoconstrictor responses in the isolated kidneys of 4-month and 13- to 14-month-old WKY rats (83 ± 9 and 95 ± 6 mm Hg respectively). It caused a larger response in the 4-month-old than in the 13-14-month-old SHR (139 ± 9 and 116 ± 8 mm Hg respectively). At 4 months of age, the response to AII was greater in kidneys from SHR than in those from WKY. The amplitude of the vascular responses to AII decreased abruptly when the administration of the peptide was repeated after 6 minutes; on repeated administration, with a dosing cycle of 6 minutes, no significant further changes were noted. There were no differences between age groups or between normotensive and hypertensive rats as regards tachyphylaxis to AII (fig. 4).

Cross-Tachyphylaxis Experiments

The response to AII in kidneys of 4-month-old hypertensive rats was similar before and after induction of maximal tachyphylaxis to 5-hydroxytryptamine; kidneys of 5-month-old SHR made tachyphylactic to AII did not exhibit a decreased response to 5-hydroxytryptamine (fig. 5). Similar results were obtained in 13-14-month-old SHR, and in both 4- and 13-14-month-old normotensive rats (table 2).

Calcium Concentration and Tachyphylaxis

The effect of low Ca++ was investigated in kidneys taken from 5-month-old SHR; the two kidneys of the same rats were perfused simultaneously with either control solution or low Ca++ solution. The vasoconstrictor response to nerve stimulation was significantly lower in low Ca++ than in control solution (18 ± 2 and 180 ± 16 mm Hg respectively), as was that to AII (56 ± 7 and 136 ± 15 mm Hg respectively). By contrast, the response to 5-hydroxytryptamine was comparable in both Ca++ concentrations (220 ± 10 and 230 ± 16 mm Hg). The time course of the tachyphylaxis to both 5-hydroxytryptamine and AII was not significantly affected by decreasing the Ca++ concentration (fig. 6).

Discussion

Increased reactivity of the vascular system to vasoconstrictor influences may contribute to the initiation or maintenance of hypertension. In mature SHR, the vascular reactivity to exogenous norepinephrine, AII, and barium chloride is increased, but the degree of increase is more pronounced for 5-hydroxytryptamine. Reactivity of the vascular

**TABLE 1. Vascular Reactivity to Increasing Doses of 5-Hydroxytryptamine in Isolated Rat Kidneys**

<table>
<thead>
<tr>
<th>Maximal response</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.5 mos</td>
</tr>
<tr>
<td>WKY</td>
<td>6.99 ± 0.03*</td>
</tr>
<tr>
<td></td>
<td>6.41 ± 0.03*</td>
</tr>
<tr>
<td>SHR</td>
<td>7.12 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>6.46 ± 0.08</td>
</tr>
</tbody>
</table>

Data shown are mean ± SEM for the experiments illustrated in figure 1. *Significantly different from 6 and 12 months of age (p < 0.05). †Significantly different from normotensive rats (p < 0.05).
TABLE 2. Vascular Responses of Isolated Kidneys of the Rat to 5-Hydroxytryptamine and Angiotensin II after Inducing Tachyphylaxis to the Other Agonist

<table>
<thead>
<tr>
<th>Dose</th>
<th>Control response before inducing tachyphylaxis to angiotensin II (mm Hg)</th>
<th>After inducing tachyphylaxis to angiotensin II (% of control)</th>
<th>Control response before inducing tachyphylaxis to 5-hydroxytryptamine (mm Hg)</th>
<th>After tachyphylaxis to 5-hydroxytryptamine (% of control response)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-hydroxytryptamine (2 × 10^-6 g)</td>
<td>156 ± 8</td>
<td>168 ± 9</td>
<td>205 ± 13*</td>
<td>180 ± 11</td>
</tr>
<tr>
<td>Angiotensin II (2 × 10^-6 g)</td>
<td>84 ± 4</td>
<td>98 ± 4</td>
<td>92 ± 5</td>
<td>104 ± 6</td>
</tr>
</tbody>
</table>

Data expressed as means ± SEM for six rats in each group.
*Difference with control rate of the same age group is statistically significant (p < 0.05).

System is influenced by age, as shown in normotensive animals for the alpha- and beta-adrenergic effects of catecholamines, for cyclic nucleotides, phosphodiesterase-inhibitors, potassium-chloride, and 5-hydroxytryptamine.7-10 Our present study investigated the effect of aging on the hyperreactivity of the SHR kidney to 5-hydroxytryptamine, using standard techniques.1-3 It demonstrates that the vascular-reactivity to both low and high doses of 5-hydroxytryptamine is increased in kidneys from SHR compared with age-matched normotensive rats. This pattern of vascular hyperreactivity can be explained by a combination of morphological and functional mechanisms contributing to the increased vascular responses.4-11

Sensitivity to 5-hydroxytryptamine decreases from 3.5 to 6 months of age in normotensive rats, while the amplitude of the maximal response increases from 3.5 to 12 months of age. This increase is correlated with a small but significant rise in SPB. These data suggest that the age-dependent depression of the vascular sensitivity to this vasoconstrictor agonist does not compensate for the structural changes of the vasculature secondary to the small increase in BP with aging. In hypertensive animals, no change in vascular reactivity to 5-hydroxytryptamine was seen between the different age groups. The progressive depression of the vascular response to repeated injections of high doses of 5-hydroxytryptamine was less pronounced in hypertensive rats compared with age-matched normotensive animals.

FIGURE 5. Absence of cross tachyphylaxis between angiotensin II and 5-hydroxytryptamine in isolated perfused kidneys of 4-month-old SHR. Upper graph: Response to 2 × 10^-6 g angiotensin II (shaded symbols) is obtained before (left) and after (right) evoking full tachyphylaxis to 5-hydroxytryptamine (middle). Lower graph: Similar experiment with maximal responses to 2 × 10^-6 g 5-hydroxytryptamine (black symbols) before and after evoking tachyphylaxis to angiotensin II. The maximal responses are shown in absolute values (mean ± SEM); the rate of tachyphylaxis is expressed as percent of the first response and shown as mean.
Figure 6. Absence of significant effect of perfusion with low Ca\textsuperscript{2+} solution on the rate of tachyphylaxis to 5-hydroxytryptamine (5-HT; left) and angiotensin II (Angio-II; right) in isolated perfused kidneys of 5-month-old SHR. Vascular responses are expressed as percent of the amplitude to the first response, and shown as means ± SEM.

Our present study demonstrates that alterations in the rate of development of tachyphylaxis and increased reactivity to 5-hydroxytryptamine represent a specific alteration of vascular smooth muscle of the SHR. Since qualitatively similar changes also occur in older normotensive animals, it can be speculated that the decreased tachyphylaxis to the monoamine observed in the young SHR may be due to premature functional aging of the kidney vasculature, chronically exposed to a high BP. Although earlier evidence did not support the view that alterations in sensitivity to 5-hydroxytryptamine play a direct role in the maintenance of the high BP in the SHR, more recent work demonstrates that doses of a selective 5-HT\textsubscript{1} serotonergic antagonist, which do not affect adrenergic responsiveness in the SHR, decrease arterial blood pressure more than in normotensive control rats. Taken in conjunction with the present and earlier\textsuperscript{1} demonstration of increased renal vasoconstrictor reactions and delayed tachyphylaxis to 5-hydroxytryptamine, and in particular with the positive correlation between SBP and vascular responsiveness to the monoamine, these observations prompt the suggestion that in the intact SHR increased or normal levels of 5-hydroxytryptamine in the vicinity of the vascular smooth muscle cells may contribute to the etiology of the hypertensive process.

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