Decreased Release of Norepinephrine in the Isolated Kidney of the Adult Spontaneously Hypertensive Rat

PAUL M. VANHOUTTE, M.D., DAN BROWNING, M.D., EDMOND COEN, M.D., TONY J. VERBEUREN, M.D., LUDO ZONNEKEYN, M.D., AND MICHAEL G. COLLIS, M.D.

SUMMARY Renal resistance vessels of the mature spontaneously hypertensive rat (SHR) exhibit an increased reactivity to exogenous norepinephrine, but a normal response to renal nerve stimulation. This difference could be due either to depression of the exocytotic process or to accelerated disposition of the released transmitter. We compared the overflow of norepinephrine in isolated perfused kidneys from adult SHR and normotensive rats. After previous incubation with \(^{3}H\)-norepinephrine, renal nerve stimulation caused smaller increases in the overflow of intact tritiated transmitter and its metabolites in kidneys from SHR than in those from normotensive controls. A similar difference was found when the amounts of endogenous norepinephrine were measured radioenzymatically. The tissue content of norepinephrine was comparable in kidneys from both hypertensive and normotensive animals. The uptake of \(^{3}H\)-norepinephrine was similar in the kidneys from SHR and normotensive controls; cocaine caused a comparable depression of the \(^{3}H\)-uptake in both groups. These data indicate that in the adult SHR the exocytotic release of norepinephrine is depressed, which then explains the normal vasoconstrictor response to renal nerve stimulation despite the increased responsiveness of the vascular smooth muscle cells to norepinephrine.

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KEY WORDS • adrenergic neuroeffector interaction • endogenous norepinephrine • \(^{3}H\)-norepinephrine • neuronal uptake • renal nerve stimulation • spontaneous hypertension

In the isolated Tyrode-perfused kidney of the young spontaneously hypertensive rat (SHR), the vasoconstrictor response to periartrial nerve stimulation is larger than that observed in kidneys from normotensive control animals, at a time when the responsiveness to exogenous norepinephrine is comparable in both groups; the greater response to nerve stimulation is paralleled by a greater than normal overflow of endogenous norepinephrine. These observations demonstrate that at the early stages of hypertension, the adrenergic nerve endings release more transmitter in the SHR than in normotensive rats. By contrast, in the adult SHR, the vasoconstrictor response of the isolated Tyrode-perfused kidney to nerve stimulation is comparable to that obtained in kidneys from normotensive animals of the same age, although the vasoconstrictions induced by exogenous norepinephrine are larger in the former. This difference could be explained either by a decreased release of adrenergic neurotransmitter in the adult SHR, or by more active disposition of the liberated norepinephrine. The present experiments were designed to test these hypotheses.

Methods

Male SHR (IFFA/CREDO, L'Arbreste, France), 6 months of age, Kyoto Wistar, and inbred Wistar rats were used in this study. Systolic blood pressure was measured by an indirect tail cuff method in unanesthetized rats. Each group of control rats was made up of equal numbers of animals from the two normotensive strains. Control animals of similar ages were weight-matched with the SHR.
Isolated Perfused Kidney Preparation

Rats were anesthetized with pentobarbitone-sodium (50 mg/kg, i.p.) and the abdomen opened by midline incision. The right renal artery, the left spermatic artery, and the left suprarenal artery were ligated. A cannula was inserted into the aorta rostral to the renal arteries and positioned with its tip adjacent to the left renal artery. The cannula was secured by a ligature, and the aorta caudal to the left renal artery was tied off and cut. The left kidney and cannulated segment of the aorta were removed from the animal and placed in a chamber containing Tyrode’s solution at 37°C. This procedure interrupted the blood supply to the kidney for 15 to 30 seconds while the cannula was inserted, and before perfusion with Tyrode’s solution was commenced.

The isolated kidney was perfused with Tyrode’s solution (37°C), using a constant flow roller pump (Gilson, Minipuls II, Middleton, Wisconsin). The perfusion rate was set at 6.2 ml/min; earlier work has shown that this rate ensures optimal perfusion conditions for the kidney of both normotensive and hypertensive rats. Renal vasoconstrictor responses were recorded as increases in perfusion pressure, downstream from the pump. Electrodes placed around the renal artery were used to stimulate the renal nerves (Janssen Scientific Instruments, Universal stimulator). The stimulation (2, 6, or 16 Hz) was applied during 2 or 4 minutes.

Measurement of \(^3\)H-Efflux

The kidneys were perfused for 30 minutes with Tyrode’s solution containing \(1.5 \times 10^{-7}\) (\(7^-\)H)-1-norepinephrine (specific activity 8.8 Ci/mmoles; American). After a washout period of 30 minutes, the perfusate was collected at 30-second intervals into cooled tubes containing protective agents and carriers for the determination of total radioactivity. The samples obtained 4 minutes before and during the stimulation period were pooled for the column chromatographic separation of \(^3\)H-norepinephrine from its major metabolites (3, 4-dihydroxymandelic acid (DOMA); 3, 4-dihydroxyphenylglycol (DOPEG); 3-methoxy-4-hydroxyphenylglycol (MOPEG); normetanephrine (NMN); and 3-methoxy-4-hydroxymandelic acid (VMA)), using a method described in detail elsewhere.

Analysis of the Results

Data are expressed as mean ± SEM throughout this paper. Significant differences \((p < 0.05)\) between means were evaluated using Student’s \(t\) test for paired or unpaired observations. On each experimental day, kidneys from SHR and control rats were perfused in parallel with the same solutions.

Results

The systolic blood pressure of the SHR was significantly greater than that of the control rats; the body weights and the kidney weights of SHR and control rats were not significantly different (table 1). No significant differences were observed between Kyoto-Wistar and inbred Wistar rats in any of the experimental conditions tested.
TABLE 1. Blood Pressure, Body Weight, and Kidney Weight

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SHR</th>
<th>No.</th>
<th>Control rats</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>210.5 ± 2.0*</td>
<td>24</td>
<td>147.2 ± 1.8</td>
<td>24</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>399.6 ± 15.5</td>
<td>19</td>
<td>429.6 ± 16.64</td>
<td>19</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>1.41 ± 0.08</td>
<td>19</td>
<td>1.33 ± 0.07</td>
<td>19</td>
</tr>
</tbody>
</table>

Data are mean values ± SEM.
*Significantly different from control (p < 0.05).

Perfusion Pressure and ¹H-Efflux

Renal nerve stimulation (6 Hz for 4 minutes) was applied to kidneys from two groups of animals each comprised of equal numbers of SHR and control rats. The constrictor responses to nerve stimulation were of comparable amplitude in both groups (fig. 1 upper). The perfusate was collected at 0.5-minute intervals, before, during, and after the stimulation period to estimate the total radioactivity. Renal nerve stimulation caused a significant increase in total ¹H-efflux in both groups. This increase in the kidneys from SHR was smaller than that in preparations from normotensive animals; this difference was significant toward the end of the stimulation period (fig. 1 lower).

Column chromatographic analysis was performed on perfusate samples collected immediately before the stimulation period (fig. 1, sample A) during the stimulation period (sample B), immediately after the stimulation (sample C), and after a 20-minute washout period (sample D). The results are shown in table 2. The only significant difference with regard to the amounts of norepinephrine and its metabolites present was that electrical stimulation caused a significantly smaller increase in ¹H-norepinephrine in the perfusate of kidneys from SHR.

Overflow of Endogenous Norepinephrine

The perfusate from kidneys of both SHR and normotensive rats was collected before and during renal nerve stimulation (2 minutes at either 2, 6, or 16 Hz). The basal efflux of norepinephrine from SHR kidneys (mean 0.11 ± 0.04 10⁻¹² mole/g/min) was not significantly different from that from control animals (mean 0.14 ± 0.05 10⁻¹² mole/g/min). In both groups, electrical stimulation evoked a frequency-dependent increase in the overflow of endogenous norepinephrine; at each frequency the efflux above the basal value induced by the stimulation was smaller in the kidneys from SHR than from normotensive control rats (fig. 2).
### Table 2. Efflux of $^3$H-Norepinephrine and Its Metabolites in Isolated Perfused Rat Kidneys

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>NE</th>
<th>DOPEG</th>
<th>DOMA</th>
<th>NMN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal efflux (sample A)</td>
<td>7.24 ± 2.01</td>
<td>8.36 ± 1.41</td>
<td>5.94 ± 0.82</td>
<td>5.75 ± 1.12</td>
</tr>
<tr>
<td>Electrical stimulation (6 Hz) (sample B)</td>
<td>1257.7 ± 135.8*†</td>
<td>81.7 ± 21.6†</td>
<td>1293 ± 19.8†</td>
<td>81.9 ± 16.9†</td>
</tr>
<tr>
<td>Basal efflux (sample C)</td>
<td>283.5 ± 26.7†</td>
<td>39.8 ± 6.32†</td>
<td>33.4 ± 5.01†</td>
<td>77.5 ± 19.7†</td>
</tr>
<tr>
<td>Basal efflux (sample D)</td>
<td>13.11 ± 0.87†</td>
<td>9.55 ± 1.44†</td>
<td>6.42 ± 1.08†</td>
<td>12.60 ± 3.30†</td>
</tr>
<tr>
<td>Spontaneously hypertensive rats (SHR) (n = 7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal efflux (sample A)</td>
<td>4.37 ± 1.00</td>
<td>6.17 ± 0.74</td>
<td>4.46 ± 0.77</td>
<td>5.50 ± 1.40</td>
</tr>
<tr>
<td>Electrical stimulation (6 Hz) (sample B)</td>
<td>723.3 ± 90.0*†</td>
<td>46.03 ± 9.21†</td>
<td>87.3 ± 13.3†</td>
<td>59.2 ± 10.5†</td>
</tr>
<tr>
<td>Basal efflux (sample C)</td>
<td>230.6 ± 49.8†</td>
<td>30.96 ± 4.67†</td>
<td>36.8 ± 5.80†</td>
<td>52.9 ± 15.8†</td>
</tr>
<tr>
<td>Basal efflux (sample D)</td>
<td>9.70 ± 1.49*</td>
<td>8.25 ± 1.17†</td>
<td>4.43 ± 0.84†</td>
<td>9.31 ± 2.24†</td>
</tr>
</tbody>
</table>

Values shown as means ± SEM and expressed as 10$^3$ DPM/4 min/g kidney. NE = norepinephrine; DOPEG = 3,4-dihydroxyphenylglycol, DOMA = 3,4-dihydroxymandelic acid; NMN = normetanephrine; MOPEG = 3-methoxy-4-hydroxyphenylglycol; VMA = 3-methoxy-4-hydroxymandelic acid.

*Value significantly different from control rats. (Student’s t test for unpaired observations; p < 0.05).
†Value significantly different from preceding value (Student’s t test for paired observations; p < 0.05).

### Content of Norepinephrine

There was no significant difference between the norepinephrine content of kidneys from SHR and normotensive rats (246.7 ± 36.3 and 255.2 ± 53.2 ng/g wet weight, respectively; n = 6).

### Uptake of $^3$H-Norepinephrine

There was no significant difference in the uptake of radioactivity between kidneys from SHR and normotensive rats, perfused for 30 minutes with either $1.5 \times 10^{-7}$ or $1.5 \times 10^{-8}$M $^3$H-norepinephrine; cocaine ($10^{-8}$ or $3 \times 10^{-8}$M) caused a comparable inhibition of the $^3$H-uptake in both groups (fig. 3).

In kidneys from four SHR and four normotensive controls, perfusion with $1.5 \times 10^{-7}$M $^3$H-norepinephrine for 10 minutes resulted in a $^3$H-uptake which averaged 1569 ± 44 and 1732 ± 132 dpm/g kidney, respectively; in kidneys perfused with a solution containing $3 \times 10^{-8}$ M cocaine, the $^3$H-uptake averaged 253 ± 31 and 281 ± 48 dpm/g kidney for the SHR and the normotensive rats, respectively. The differences between the two groups were not statistically significant.

### Figure 3. Uptake of radioactivity in kidneys from adult normotensive (Wistar and Wistar-Kyoto, WKY) and SHR rats, perfused for 30 minutes with solution containing $1.5 \times 10^{-8}$M (left) or $1.5 \times 10^{-7}$M (right) $^3$H-norepinephrine. Open columns = experiments performed in control solution. Dotted bars = experiments performed in presence of $10^{-4}$ M cocaine. Hatched bars = experiments performed in presence of $3 \times 10^{-8}$M cocaine. Data are shown as means ± SEM (n = 6). Asterisks indicate that the difference from the preceding value is significant (p < 0.05). No significant differences were observed between kidneys from SHR and normotensive animals.
Table 2. (Continued)

<table>
<thead>
<tr>
<th>MOPEG</th>
<th>VMA</th>
<th>Total radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.61 ± 1.20</td>
<td>2.73 ± 0.42</td>
<td>43.05 ± 7.20</td>
</tr>
<tr>
<td>81.0 ± 12.2 †</td>
<td>8.63 ± 1.65 †</td>
<td>2068 ± 207 †</td>
</tr>
<tr>
<td>47.4 ± 8.06 †</td>
<td>4.42 ± 0.88 †</td>
<td>613.7 ± 75.0 †</td>
</tr>
<tr>
<td>4.54 ± 1.37 †</td>
<td>2.63 ± 0.49 †</td>
<td>55.8 ± 6.58 †</td>
</tr>
<tr>
<td>11.93 ± 1.61</td>
<td>1.77 ± 0.37</td>
<td>37.53 ± 4.63</td>
</tr>
<tr>
<td>61.5 ± 7.9 †</td>
<td>6.98 ± 1.07 †</td>
<td>1302 ± 168.2 †</td>
</tr>
<tr>
<td>40.0 ± 5.41 †</td>
<td>3.35 ± 0.47 †</td>
<td>500.7 ± 84.0 †</td>
</tr>
<tr>
<td>10.1 ± 1.83 †</td>
<td>1.57 ± 0.30 †</td>
<td>47.2 ± 5.45 †</td>
</tr>
</tbody>
</table>

Discussion

Our present experiments in which the appearance of both endogenous and labeled norepinephrine were measured demonstrate that in the isolated Tyrode-perfused kidney of the adult SHR smaller amounts of norepinephrine overflow to the perfusate during sympathetic nerve stimulation than is the case in normotensive control rats. Since this coincides with a normal vasoconstrictor response to the stimulation, at a time that the constrictor response to exogenous norepinephrine is augmented,7 the decreased overflow of norepinephrine must reflect a lower concentration of the adrenergic neurotransmitter in the junctional cleft rather than a decreased hindrance to diffusion towards the extracellular space.4,7,16 Theoretically, a decreased junctional concentration of norepinephrine could be due to reduced exocytotic release or to faster disposition of the released transmitter.4,7,16 Experiments with the inhibitor of neuronal uptake, cocaine, favor the interpretation that the "normal" vasoconstriction to renal nerve stimulation observed in the Tyrode-perfused isolated kidney of the SHR is due in part to hyperactivity of the neuronal uptake mechanism.7 Both biochemical determinations and functional studies have shown that, in the tail artery of the hypertensive strain, the neuronal uptake of norepinephrine is greater than that in arteries from normotensive animals;4,9 this appears also to be the case in smaller mesenteric blood vessels.17,18 However, the present experiments, in which the overflow of the metabolites of norepinephrine was measured, do not support the concept that accelerated disposition lowers the effective junctional concentration of intact transmitter during nerve stimulation, since the appearance of the metabolites, and in particular of DOPAC which reflects the degree of neuronal uptake,4,16,18,20 was not augmented in the kidneys from the SHR. The experiments where the cocaine-sensitive uptake of "H-norepinephrine was determined after different periods of incubation with the labeled transmitter demonstrate that in the SHR kidney, unlike in the tail artery of the same strain,9 the kinetics of the neuronal uptake of norepinephrine in quiescent preparations are comparable to those observed in normotensive animals.

Our present experiments thus indicate that, in the SHR, the amount of transmitter liberated per impulse into the junctional cleft is smaller than in kidneys from normotensive rats; they indirectly confirm that the activity of the disposition pathways for the adrenergic neurotransmitter plays little role in determining the effector response during activation of the exocytotic process.4,7,16 We have reported7 that cocaine causes a greater potentiation of the vasoconstrictor response evoked by adrenergic nerve stimulation in kidneys from SHR than from normotensive control rats. Since the concentration of cocaine used in these earlier experiments causes comparable reduction in the uptake of "H-norepinephrine in quiescent kidneys of SHR and normotensive rats, our present results suggest that this effect may have been a consequence of an effect of cocaine not related to blockade of neuronal uptake. One possibility could be that the local anesthetic effect, presumed to occur with high concentrations of the substance,16 is more pronounced on the adrenergic nerves of the normotensive control animals than on those of the SHR.

In the in situ blood autoperfused kidney of the anesthetized SHR (3 to 4 months of age), the basal resistance to flow is increased, and the vasoconstrictor response to angiotensin II is comparable to that seen in normotensive animals, while that to norepinephrine is less.51 By contrast, in the Tyrode-perfused isolated SHR kidney, the basal resistance to flow is normal, but the vasoconstrictor responses to both angiotensin II and norepinephrine are greater than those noted in preparations from normotensive rats.5 In the Tyrode-perfused kidney, the use of protein-free medium may alter the autoregulatory behavior and reduce the responsiveness of the vascular smooth muscle cells;51 this could explain the normal basal perfusion pressure, but not the greater than normal responsiveness to angiotensin II, 5-hydroxytryptamine, and norepinephrine.7 Under conditions of pump perfusion, renal endogenous substances may be released that modify the responsiveness of vascular smooth muscle cells of the renal resistance vessels;51 if so, it would be surprising that these endogenous substances affect the response to nerve stimulation differently than that to the administration of exogenous transmitter, and even more so that they augment the constrictor responses to 5-hydroxytryptamine more than to norepinephrine and angiotensin II.7

The difference between Tyrode-perfused kidneys of normotensive and SH rats can hardly be attributed to the perfusion technique used, since in identical experimental conditions the vasoconstrictor responses of kidneys from renal hypertensive rats are comparable...
to, but those to angiotensin II larger than, those obtained in kidneys from control animals. On the other hand, in the blood autoperfused kidney, the vascular responsiveness may be influenced by substances released from blood constituents in the extracorporeal circuit, or by the anesthetic agent used; the influence of the latter is obvious from the marked decrease in arterial blood pressure that occurs during anesthesia. Estimation of the true dose-response relationships to exogenous norepinephrine is made difficult in the face of circulating catecholamines and the persistence of sympathetic tone. Furthermore, maximal contractile responses of the vascular smooth muscle cells to vasoconstrictor agonists and nerve stimulation are impossible to determine without altering the general hemodynamic behavior of the animal. Whatever the reasons may be for the differences observed in the two experimental models, they agree in that the vasoconstrictor response to sympathetic nerve stimulation is similar in kidneys from adult SHR and normotensive rats. Our present study, where the overflow of released transmitter was actually measured, provides direct evidence that, to obtain this "normal" renal constrictor response during activation of the sympathetic nerves, less liberated norepinephrine is required.

Our present study does not explain why, unlike in the young SHR, the release of transmitter is reduced in the isolated Tyrode-perfused kidney of the adult animal, although the similarity in endogenous content with that obtained in control rats makes it unlikely that it reflects a relative decrease in the density of innervation. It has been suggested that, since the release of norepinephrine by peripheral nerve endings of the vascular wall is greater than normal in the young SHR, this enhancement of the exocytotic process may be one of the factors causing hypertension; this would be of particular importance in view of the increased sympathetic traffic to the cardiovascular periphery which has been described also at the early stage of the disease. If it is assumed that the experiments on the Tyrode perfused kidneys of adult SHR reflect the behavior of the adrenergic neurotransmission in the intact animal, the present study implies that, if the release of norepinephrine in the blood vessel wall plays a role in maintaining the increased peripheral resistance in the chronic stage of the hypertensive process, this can only be attributed either to such central increases in sympathetic traffic, or to the presence of locally produced or circulating facilitators of the exocytotic release of norepinephrine.

References

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