Catecholamines in Discrete Kidney Regions
Changes in Salt-Sensitive Dahl Hypertensive Rats

JULIO FERNANDEZ-PARDAL, M.D., AND JUAN M. SAAVEDRA, M.D.

SUMMARY Steady state levels of catecholamines (dopamine, norepinephrine, and epinephrine) were measured by the use of radioenzymatic techniques in discrete areas of the kidney (outer and inner cortex, outer and inner medulla) dissected by a “punch” technique from frozen kidney sections of salt-sensitive (DS) and salt-resistant (DR) Dahl rats fed low or high salt diet. All three catecholamines were present in all areas of the kidney examined. There were gradients of concentrations of each catecholamine in different kidney areas. Renal medullary areas contained proportionally more dopamine than cortical areas. The proportion of epinephrine with respect to the total catecholamine content was relatively high in the inner medulla. Genetic factors and the amount of dietary salt influenced the catecholamine content in specific kidney areas, and these changes were different according to the area considered. DS rats when fed a high salt diet presented increased systolic blood pressure but no increased levels of dopamine in the inner medulla nor of norepinephrine in the outer medulla and outer cortex. Results suggest that either the uptake, release, storage, synthesis, or catabolism of kidney catecholamines is altered in Dahl salt-sensitive (DS) hypertensive rats and suggest specific roles for each catecholamine in discrete areas of the kidney.

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KEY WORDS • kidney • catecholamines • dopamine • norepinephrine • epinephrine • salt-sensitive hypertension • Dahl rats • micropunch dissection • genetic hypertension

SYMPATHETIC nerves innervating the kidney play a physiological role in renal function, probably related to their influence in the regulation of renal blood flow, glomerular filtration rate, renin secretion, and reabsorption of sodium and fluids.1-4 There is a sympathetic innervation of the juxtaglomerular apparatus, which is related to the regulation of renin secretion.1-3 Both the stimulation of the renal nerves and infusion of norepinephrine produce a cortical-renal blood flow redistribution.4 The sympathetic tubular innervation plays a role in the proximal tubular reabsorption of sodium and fluids.5, 6

Both dopamine and norepinephrine are involved in the regulation of renal function. The renal nerves release dopamine as well as norepinephrine,7 and the denervation of the kidney markedly alters the urinary excretion of both catecholamines.7 Whereas a direct correlation exists between sodium intake or excretion and urinary dopamine excretion, the correlation between these parameters and the excretion of norepinephrine is a negative one.7, 8 These observations suggested the possibility of specific and different roles for dopamine and norepinephrine regarding the regulation of sodium excretion by the kidney.

Sodium intake can markedly affect not only the excretion of catecholamines in the urine7-9 but also the function of the peripheral nervous system, by altering the nerve activity and/or the storage and release of neurotransmitters.10-12 Therefore, while catecholamines released from sympathetic renal nerves may regulate sodium excretion, sodium, in turn, may regulate sympathetic activity and catecholamine release and excretion by the kidney.

The kidney has long been known to be involved in the regulation of blood pressure as well as in the pathogenesis of hypertension. In addition to the role of humoral factors of kidney origin, the renal nerve and kidney catecholamines play a role in different forms of hypertension.13-18

It was therefore of interest to study kidney catecholamines in an animal model in which the interplay of genetic factors and dietary factors such as the salt content in the diet could be independently monitored. Such a model was first described by Dahl et al.19 in 1962, who found that selective inbreeding of Sprague Dawley rats resulted in the development of two lines, one of which rapidly developed hypertension when subjected to a high salt intake (DS) and another that was resistant to the hypertensinogenic effect of a high salt diet (DR). In this model, genetic alterations in the reabsorption of sodium in the kidney of the DS rats have been described,20, 21 and a role for the peripheral sympathetic system in the pathogenesis of the hypertension is well documented.22, 23

In addition, the α1 and α2 adrenergic receptor densities have been demonstrated to be higher in kidney homogenates of DS rats when compared to DR rats, and α2 receptor density increased in DS, but not in DR rats, when submitted to a high salt diet.24

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Recent reports of specific localization of $\alpha_2$-adrenoreceptors in discrete renal areas\(^{23}\) prompted us to develop a dissecting technique to isolate tissue from specific areas of the rat kidney, so that catecholamines could be studied in selected kidney areas.

Methods

Animals

Male DS rats and DR rats were obtained from Brookhaven National Laboratory, Upton, New York. Each rat line was divided into two groups of eight animals each. One group from each line was fed 0.45% NaCl chow from weaning until the age of 12 weeks. The other groups were fed high (8%) NaCl chow during the same period. Water was provided for drinking ad libitum.

Blood Pressure Determinations

Systolic blood pressure was measured in unanesthetized rats by tail plethysmography, using a programmed electromyogramanometer (Narco Biosystems, Houston, Texas, Model PE-500) 1 week before sacrifice as previously described.\(^{26}\)

Dissection of Specific Kidney Areas

The animals were killed by decapitation when 12 weeks of age, between 09.00 and 10.00 hours; the kidneys were removed immediately and rapidly frozen on microtome specimen holders on dry ice. Serial frontal sections of 300 $\mu$m thickness were cut at $-10^\circ$C in a microtome cryostat. Specific kidney areas were located under a stereomicroscope and punched out by the use of a stainless steel hollow needle of 1 mm inner diameter (fig. 1). Specific kidney areas are designated: inner medulla, outer medulla, inner cortex, and outer cortex, as previously described by Sternberg et al.\(^{27}\) Each area was dissected in two consecutive kidney sections, and a total of four punches were used for analysis of catecholamines.

Determination of Catecholamine Levels

Tissues from single rats were homogenized in microhomogenizers containing 60 $\mu$L of 0.1 N perchloric acid, and a 5 $\mu$L aliquot of the homogenate was removed for protein determination.\(^{28}\) After a centrifugation at 5000 $g$ for 20 minutes, a 30 $\mu$L aliquot of the supernatant was taken to assay dopamine, norepinephrine, and epinephrine by a modification of previously described isotopic radioenzymatic assays.\(^{29-31}\) This technique was based upon the use of the partially purified enzyme catechol-0-methyltransferase to transfer a tritium-labeled methyl group from S-adenosyl-L-methionine to the catecholamines to form radioactive 0-methyl catecholamine derivatives. The radioactive products were then extracted into toluene-isoamylalcohol (3:2, v/v) and separated by thin layer chromatography.

Statistical Analysis

Results were analyzed for the presence of a gradient of catecholamine concentrations within the kidney (one-way analysis of variance (ANOVA) and Student's Newman-Keuls test for comparison of individual means) and for the differences between the DR and DS lines ($F_A$), the effects of high salt diet ($F_B$), and the differential effects of the salt diet on each of the two lines (interaction: $F_{AB}$) (two-way ANOVA).\(^{32}\)

### Table 1. Total Catecholamine Content, Percent Contribution of Each Catecholamine, and Norepinephrine/Dopamine Ratio in Specific Areas of the Kidney in Dahl Rats

<table>
<thead>
<tr>
<th>Kidney area</th>
<th>Rat group</th>
<th>CA</th>
<th>DA</th>
<th>E</th>
<th>NE/DA ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner medulla</td>
<td>DR low salt</td>
<td>481</td>
<td>45</td>
<td>11</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>DR high salt</td>
<td>1248</td>
<td>29</td>
<td>4</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>DS low salt</td>
<td>1125</td>
<td>52</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>DS high salt</td>
<td>1096</td>
<td>38</td>
<td>12</td>
<td>50</td>
</tr>
<tr>
<td>Outer medulla</td>
<td>DR low salt</td>
<td>226</td>
<td>36</td>
<td>1</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>DR high salt</td>
<td>439</td>
<td>30</td>
<td>0</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>DS low salt</td>
<td>799</td>
<td>30</td>
<td>2</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>DS high salt</td>
<td>670</td>
<td>37</td>
<td>6</td>
<td>57</td>
</tr>
<tr>
<td>Inner cortex</td>
<td>DR low salt</td>
<td>1170</td>
<td>13</td>
<td>0.5</td>
<td>86.5</td>
</tr>
<tr>
<td></td>
<td>DR high salt</td>
<td>1153</td>
<td>12</td>
<td>1</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>DS low salt</td>
<td>1470</td>
<td>17</td>
<td>2</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>DS high salt</td>
<td>1255</td>
<td>22</td>
<td>8</td>
<td>70</td>
</tr>
<tr>
<td>Outer cortex</td>
<td>DR low salt</td>
<td>1646</td>
<td>23</td>
<td>3</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>DR high salt</td>
<td>2280</td>
<td>16</td>
<td>2</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>DS low salt</td>
<td>1960</td>
<td>19</td>
<td>3</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>DS high salt</td>
<td>1768</td>
<td>30</td>
<td>6</td>
<td>64</td>
</tr>
</tbody>
</table>

Results are expressed in picograms per mg of protein. Total catecholamines represent the sum of individual catecholamine levels represented in figures 2, 3, and 4. CA = catecholamines, DA = dopamine, NE = norepinephrine, E = epinephrine.
Results

Relative Contribution of Each Catecholamine to the Total Catecholamine Content

The total concentration of kidney catecholamines and the relative amount of each catecholamine depended on the kidney area for all groups of rats studied (table 1). Higher total catecholamine levels were found in the outer cortex, and lower values were always present in the outer medulla.

Medullary areas (both inner and outer medulla) showed higher dopamine levels than cortical areas. In medullary areas of all groups studied, dopamine represented between 29% and 52% of the total catecholamine content, with a norepinephrine-to-dopamine ratio between 0.99 to 2.3. In contrast, in cortical areas dopamine levels were only 12% to 30% of the total catecholamine content, and the norepinephrine-to-dopamine ratio was higher, from a minimum of 2.1 to a maximum of 7.1 (table 1).

Epinephrine concentrations were lower than those of dopamine and norepinephrine in all areas studied. Highest levels of epinephrine were found in the inner medulla, where this amine represented from 4% to a maximum of 12% of the total catecholamine content (table 1).

Distribution of Catecholamines in Specific Areas of the Kidney and Influence of Genetic and Dietary Factors

Dopamine

A significant gradient in dopamine concentrations was detected in DR rats fed low or high salt diets. Lowest values were present in the outer medulla and highest values in the outer cortex (fig. 2). Dopamine levels in the outer medulla and inner cortex differed significantly between the two lines; DS rats had higher dopamine levels in these areas than DR rats.

There was evidence of a line × salt interaction, restricted to the inner medulla region; a high salt diet resulted in higher dopamine levels only in DS rats. The dopamine levels were not modified by a high salt diet in DR or DS rats in all other kidney areas studied (fig. 2).

Norepinephrine

A significant gradient in norepinephrine concentrations was detected in all kidney areas studied. Lower norepinephrine concentrations were found in both inner and outer medullary areas, when DS and DR rats were submitted to low salt diet (fig. 3). Under a high salt diet, however, a significant gradient in norepinephrine concentrations was evident for both DR and DS rats only between the outer cortex (highest values) and the outer medulla (lowest values).

The differences in norepinephrine concentrations between the two lines, and after changes in the dietary salt content were highly dependent on the kidney area examined. A significant difference in norepinephrine concentrations between the two lines occurred only in the outer medulla, with DS rats presenting higher levels than DR rats. A high salt diet significantly increased the norepinephrine concentrations only in the inner medulla of DR rats. A line × salt interaction was observed in the outer medulla and outer cortex, where DR rats showed increased, and DS rats decreased, norepinephrine levels after a high salt diet.

Epinephrine

Large differences in epinephrine concentrations were found in all discrete areas of the kidney, with a distinct, statistically significant gradient of concentrations for DR rats fed low or high salt diets and for DS rats fed low salt diet. Significantly lower epinephrine levels were found in the outer medulla in these three groups. A tendency for lower epinephrine concentrations was also observed in the outer medulla in hyper-
tensive DS rats under a high salt diet, but the results did not attain statistical significance (fig. 4).

Epinephrine levels differed significantly between DR and DS rats in the four rat kidney areas studied; in all cases, DS rats had higher epinephrine levels than DR rats. The epinephrine levels were increased by a high salt diet in the outer medulla and inner cortex, with evidence of a line × salt interaction, since the high salt diet increased the amine levels only in the DS rats and not in DR rats. A tendency for an increase in epinephrine concentrations in hypertensive DS rats under a high salt diet was also observed in the inner medulla and outer cortex, but the results did not attain statistical significance.

Discussion

To our knowledge this is the first demonstration of the existence of concentration gradients for all three catecholamines (dopamine, norepinephrine, and epinephrine) in different kidney regions. In renal medullary areas (inner and outer medulla), the relative concentrations of dopamine with respect to norepinephrine are much higher than in cortical areas, suggesting a specific role for dopamine, besides nor-epinephrine, in the renal medulla. The renal medulla could be of one the sites of action for the natriuretic effect of dopamine, since it contains the collecting ducts, which are powerful regulators of sodium excretion. In cortical areas, the norepinephrine concentrations are always much higher than those of dopamine, suggesting a predominantly norepinephrine innervation. These results are consistent with those of Young and Kuhar, who found α-adrenoceptors exclusively localized to the cortical areas of the guinea-pig kidney.

Both dopamine and norepinephrine are located in renal sympathetic nerves and are released by renal nerve stimulation. Dopamine is located in nerve terminals at the glomerular vascular poles, whereas nor-epinephrine terminals innervate the arcuate arteries. Small intensive fluorescent cells, which may contain dopamine, are located in the perivascular plexus of renal arteries. These observations and our present findings of heterogeneous distribution of catecholamines in renal areas, indicate the existence of different catecholamine pools within the kidney and support previous evidence of specific roles for dopamine and norepinephrine.

DS rats have higher dopamine levels in the outer medulla and the inner cortex than DR rats, independent of their salt intake. Dopamine regulates the secretion of renin by the kidney, and DS rats, both when fed a high or a low salt diet, have a lower renin release than the DR rats, suggesting a relation between a probably increased formation of dopamine in the inner cortex of DS rats and the decreased renin release in this model.

The DS kidney is distinctly subnormal in sodium and water excretion. When challenged with a high salt diet, DR rats increased their blood flow to the inner medulla, an adaptation possibly important for natriure-

FIGURE 3. Norepinephrine concentrations in specific areas of the kidney of Dahl rats (means ± SEM; n = 8). DR low salt: the norepinephrine content of the inner and outer medulla is significantly lower than that of the inner or outer cortex (inner medulla = outer medulla < inner cortex = outer cortex* (F 9.67, df 3/28)). DR high salt: the norepinephrine content of the outer medulla is significantly lower than that of the outer cortex (outer medulla < outer cortex* (F 5.71, df 3/28)). DS low salt: the norepinephrine content of the inner and outer medulla is significantly lower than that of the inner or outer cortex (inner medulla = outer medulla < inner cortex = outer cortex* (F3.18, df 3/28)). DS high salt: the norepinephrine content of the outer medulla is significantly lower than that of the outer cortex (outer medulla < outer cortex* (F 5.29, df 3/28)). *p < 0.05.

Effects of line, salt, and interaction: $F_{A} = $ line (DR vs DS); $F_{B} = $ salt treatment; $F_{AB} = $ interaction (effect of salt in DR and DS). Inner medulla: salt treatment increases norepinephrine levels significantly ($F_{a} 0.87, F_{b} 5.22$, $F_{ab} 3.53$). Outer medulla: DS rats have higher norepinephrine levels; these levels are increased in DR receiving salt and decreased in DS receiving salt ($F_{a} 7.68$, $F_{b} 0.18, F_{ab} 7.06$). Inner cortex: $F_{a} 0.01, F_{b} 1.49, F_{ab} 1.29$. Outer cortex: when receiving salt, DR rats show increased norepinephrine levels, whereas DS rats show decreased levels ($F_{a} 0.19, F_{b} 0.41, F_{ab} 4.77$). *p < 0.05.

This has been postulated that, lacking their adaptive mechanism, hypertension may then occur in DS rats to accomplish natriuresis through a "pressure natriuresis" mechanism. Since dopamine is involved both in the regulation of renal blood flow and sodium excretion, an alteration in dopamine metabolism in the inner medulla could be related to the pathogenesis of salt-sensitive (Dahl) hypertension.
Effects of line, salt, and interaction: $F_A = $ line (DR vs DS); $F_B = $ salt treatment; $F_{AB} = $ interaction (effect of salt in DR and DS). In all areas, DS rats have higher epinephrine levels than DR rats. In the outer medulla and inner cortex, high salt increases epinephrine levels, but only significantly in DS rats. Inner medulla: $F_A 4.48^*, F_B 0.56, F_{AB} 0.43$. Outer medulla: $F_A 48.54^*, F_B 4.81^*, F_{AB} 8.82^*$. Inner cortex: $F_A 18.58^*, F_B 8.21^*, F_{AB} 6.09^*$. Outer cortex: $F_A 6.25^*, F_B 2.88, F_{AB} 3.10$. $p < 0.05$.

Norepinephrine also plays a role in the regulation of sodium excretion through its antinatriuretic effect, and the excretion of norepinephrine in the urine is inversely influenced by the salt intake. The failure of DS kidneys to increase norepinephrine concentrations after a high salt diet indicates that alterations in norepinephrine uptake, release, storage, synthesis, or catabolism in Dahl hypertension are operative both at medullary and cortical locations. In renal homogenates, the $\alpha_2$-adrenoceptor number has been recently found to be increased in DS rats, with respect to DR controls, and more so when the DS rats are fed a high salt diet. Norepinephrine activates $\alpha$-receptors at peritubular sites, resulting in sodium reabsorption. An alteration of the norepinephrine metabolism and/or the regulation of the $\alpha_2$-adrenoceptors could also play a pathogenic role in the salt-sensitive (Dahl) hypertension.

The present changes in steady-state levels of norepinephrine may be different from those observed in another model of hypertension, the spontaneously (genetic) hypertensive rat (SHR), where the whole kidney levels of norepinephrine were decreased when compared to the corresponding controls.

Our data show that epinephrine levels in rat kidney are determined both genetically and by salt intake. These results also suggest a participation of kidney epinephrine in salt-dependent hypertension. Epinephrine may have direct effects on the kidney similar to those of dopamine or norepinephrine, or indirect effects, through modulation of catecholamine release.

We report only steady-state levels of catecholamines, and it is not possible to know whether low or high tissue levels represent low or high rates of synthesis or low or high rates of release and metabolism of the amines. Such information could only be obtained by a direct measurement of the catecholamine turnover and/or the determination of the endogenous levels of all the principal catecholamine metabolites in each kidney area. Also, determination of steady-state levels of catecholamines can not clarify the source or location of the amines in tissues. Kidney catecholamines, as measured in our study, could represent catecholamine pools synthesized within renal sympathetic nerves, amines of other origins mainly the adrenal medulla, which are released into the circulation, taken up from the blood and stored in renal sympathetic nerves, catecholamines formed in kidney structures other than sympathetic nerves, or more probably a combination of these. In addition, there could be a contribution of circulating catecholamines, which are normally filtered through the glomeruli, actively excreted through the proximal tubule, and concentrated in the urine.

In our model, however, basal plasma levels of norepinephrine and epinephrine were not different in any of the groups studied (epinephrine: 190 ± 10, 150 ± 25, 165 ± 8, 200 ± 36 and norepinephrine: 780 ± 87, 595 ± 90, 710 ± 68, 600 ± 105 pg/ml of plasma for DR under low salt, DR under high salt, DS under low salt, and DS under high salt, respectively), indicating that kidney levels of catecholamines may not be a direct consequence of altered blood levels of the amines.

Whatever the mechanism of the changes in metabolism of catecholamines observed here, and the sources and cellular localization of the different renal catecholamine pools, our results suggest that: 1) renal levels of all three catecholamines can be regulated by both genetic as well as dietary factors; 2) this regulation differs according to the renal area considered; and 3) kidney catecholamines could play a role in the pathogenesis of the genetic, salt-dependent (Dahl) hypertension.
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