Catecholamines in Kidneys of Normotensive and Genetically Hypertensive Rats
Effects of Salt Load

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SUMMARY The tissue content of norepinephrine, dopamine, and epinephrine was determined in different zones of the kidney in normotensive Sprague-Dawley and Otago Wistar rats and in genetically hypertensive Otago Wistar rats. One kidney in each animal was chronically denervated to allow estimation of the neuronal contribution to renal catecholamine content. In all strains, the renal cortex contained negligible amounts of nonneuronal norepinephrine and dopamine, while outer and inner medullary layers contained progressively larger amounts. Nonneuronal epinephrine was distributed fairly evenly through cortex and medulla. Neuronal norepinephrine content was similar in inner and outer cortex, substantially less in outer medulla, and not discernible in inner medulla. The amounts of neuronal dopamine were consistent with its localization predominantly in noradrenergic nerves. The renal cortices of normotensive Wistar rats contained more neuronal norepinephrine and less neuronal dopamine than those of Sprague-Dawley rats, and the cortices of hypertensive Wistar rats contained slightly more norepinephrine than those of normotensive Wistar rats. In both normotensive strains, long-term salt loading decreased selectively the neuronal norepinephrine in renal cortex. By contrast, in hypertensive animals, cortical norepinephrine was not reduced by salt loading. These results indicate that the genetically hypertensive rat may have an abnormal sympathetic reflex response to increased blood volume. (Hypertension 8: 122-127, 1986)

Key Words • dopamine • norepinephrine • epinephrine • kidney • sympathetic nerves • hypertension

THE catecholamines — dopamine (DA), norepinephrine (NE), and epinephrine (EPI) — can alter the renal handling of water and electrolytes by changing renal vascular resistance and tubular reabsorption, either through effects on renin secretion or by direct effects on the tubular epithelium. In general, NE and EPI have antinatriuretic effects, while DA produces natriuresis.

Catecholamines acting within the kidney may arise from several sources. Circulating free dopa can be decarboxylated to DA by enzymic activity in the tubular epithelium, and conjugated plasma catecholamines can be deconjugated to the free amines as they pass through the renal circulation. In addition, renal sympathetic nerves supply intrarenal resistance vessels, juxtaglomerular cells, and renal tubules. While the majority of these nerves are noradrenergic in nature, recent functional, biochemical, and morphological data have indicated the existence of intrarenal nerves in which the transmitter is DA.

To understand the roles of the different catecholamines in modulating renal function, the sources of renal catecholamines and their distributions must be identified. As part of this exercise, we studied the neuronal and nonneuronal distributions of NE, DA, and EPI in cortical and medullary zones of the kidneys from two commonly used strains of normotensive experimental rats (Sprague-Dawley and Wistar) and from a genetically hypertensive Wistar strain. We also compared the effects of long-term salt loading on renal catecholamines in each strain.

Materials and Methods

All rats used were bred in our animal house. The strains involved were an outbred white Sprague-Dawley line, an inbred white Wistar line originating from the Otago Medical School, and an inbred Wistar line.
with hereditary hypertension, originating from the same genetic stock. Both male (300-400 g) and female (200-300 g) animals (age, 3-4 months) were used, and the hypertensive and normotensive Wistar rats were age-matched. All rats were fed a proprietary rat chow (quoted sodium content, 0.5%) and given water ad libitum. Long-term salt loading was performed by substituting drinking water with 0.9% normal saline for at least 6 weeks.

To confirm that blood pressure was not affected by salt loading per se, systolic blood pressures were recorded twice weekly in conscious, prewarmed normotensive (n = 10) and hypertensive (n = 10) female Otago Wistar rats, using an occlusive tail cuff and a pneumatic pulse transducer attached to a programmed electrophysgmononometer (PE-300, Narco Bio-Systems, Houston, TX, USA), which was calibrated against a mercury-in-glass manometer after each set of readings. To minimize any diurnal variations in pressure, all measurements were made between 1430 and 1700 by a technician who was not aware of the experimental protocol. Half of each group was salt loaded for 6 weeks. At the end of this time, the mean blood pressures of the normotensive animals were 124 ± 5 (control) and 121 ± 2 (salt-treated) mm Hg, and those of the hypertensive animals were 185 ± 12 (control) and 193 ± 3 (salt-treated) mm Hg.

Renal denervation was accomplished through a left flank incision with the rats under sodium pentobarbitone anesthesia (30 mg/kg i.p.) by freezing a section of the left renal artery and vein with 3-mm wide forceps dipped in liquid nitrogen, taking care to avoid the ureter. Antibiotic powder (Cicatrin) was dusted over the kidney and in the abdominal cavity, and the incision was closed with muscle sutures and skin Michel clips. The animals were allowed to recover for 6 to 8 days after the operation to ensure complete degeneration of all intrarenal nerve terminals without the possibility of sprouting nerves having time to reenter the kidney. At this time, there was no difference in size, weight, or macroscopic appearance between the control and denervated kidney.

Animals were killed with ether. The kidneys were quickly removed and placed on ice. They were blotted dry, and samples of outer cortex, inner cortex, outer medulla, and inner medulla were removed with a scalpel blade, using the zonal classification of Sterberg et al. In addition, a sample from each kidney was prepared for catecholamine fluorescence histochemistry to confirm the completeness of denervation.

The tissue samples were minced finely with scissors, weighed, and placed on ice in 2 ml of 0.1 M perchloric acid containing disodium ethylenediaminetetraacetic acid (EDTA) and sodium metabisulfite. Di-hydroxybenzylamine (Aldrich Chemical, Milwaukee, WI, USA) was added as an internal standard. After extraction for at least 30 minutes, 1 ml of solution was removed. To this, 50 mg of alumina (grade V; Sigma Chemical, St. Louis, MO, USA) was added, and the pH was adjusted to 8.6 by adding an equal volume of 1 M Tris buffer. After adsorption of catecholamines by shaking for 15 to 20 minutes, the alumina was washed three times with 0.01 M Tris buffer and the catecholamines were resorbed with 0.2 ml of 0.1 M perchloric acid containing EDTA and sodium metabisulfite. The catecholamines were separated by high-pressure liquid chromatography on a 5-μm C18 column with a citrate-phosphate mobile phase (pH 5.2), and they were assayed by oxidative electrochemistry. Recoveries were 47 ± 0.5%; typical retention times in minutes were 5 for NE, 7 for EPI, 8.5 for dihydroxybenzylamine, and 13 for DA. The minimum amount of amine that could be reliably detected was 10 pg, which corresponded to minimum detectable tissue concentrations ranging from about 1 pg/mg wet mass in outer cortex to 10 to 15 pg/mg wet mass in inner medulla. Samples in which particular amines were not detected were arbitrarily allocated the default value of 10 pg; therefore, absolute tissue concentrations are in some cases marginal over-estimates of the true values. All data were recorded with a Shimadzu CR1 programmable computing integrator (Shimadzu, Kyoto, Japan) and were adjusted for losses in processing.

Differences of means were analyzed using two-tailed paired, unpaired Student's t tests and the Mann-Whitney U test. Results are expressed as means ± SEM.

**Results**

**Catecholamines in Normotensive Rats**

Of the three catecholamines, only EPI was characteristically distributed evenly through all zones of the kidney. The EPI was entirely nonneuronal in location, as there was no difference between contents of intact and denervated kidneys, and there was no apparent difference in EPI content between rats of either sex or between Sprague-Dawley and Wistar rats. Pooled values for EPI content for all animals used (n = 39) were 19 ± 6.7 for outer cortex, 17 ± 5.4 for inner cortex, 31 ± 11.4 for outer medulla, and 137 ± 55.4 pg/mg wet mass for inner medulla. The high relative value for inner medullary content reflects distortion of the mean by two animals out of 39 in which EPI contents were far in excess of the usual range (1500-3000 vs 10-50 pg/mg). Without these values the mean for inner medulla was 45 ± 12 pg/mg wet mass.

The zonal contents of NE and DA for intact and contralateral denervated kidneys of male and female rats of the two strains examined are shown in Figure 1. It can be seen that, for both strains, the total NE content was distributed in the following manner: inner cortex = outer cortex > outer medulla > inner medulla; total DA distribution, in descending order: inner medulla > outer cortex > inner cortex > outer medulla. Comparison of data from intact and denervated kidneys demonstrated that inner medullary amines were entirely nonneuronal for both NE and DA. By contrast, nerves contributed virtually all the NE and a proportion of the DA in outer medulla and in both cortical layers. In general, no striking variations were seen between male and female animals; however,
neuronal NE was significantly greater in the outer cortex of female than of male Sprague-Dawley animals and in the inner cortex of female than of male Wistar rats (p < 0.05).

Neuronal DA values were rather greater in cortices of Sprague-Dawley than of Wistar rats (female rats, p < 0.05 for outer cortex, p < 0.01 for inner cortex; male rats, p < 0.05 for inner cortex only), while neuronal NE values were higher in the outer cortex of male and the inner cortex of female Wistar rats than in the corresponding zones of Sprague-Dawley animals (both, p < 0.01). The relative amounts of neuronal DA and NE were about 1:20 for Sprague-Dawley rats and about 1:50 for Wistar rats. The difference in this ratio was significant for inner cortex in both sexes and for outer cortex in male animals (p < 0.05).

Catecholamines in Hypertensive Rats

The zonal contents of NE and DA for male and female hypertensive rats are compared with those for the genetically related normotensive Wistar rats in Figure 1. Significant differences were found only for the male rats: the hypertensive animals showed a higher level of outer (p < 0.05) and inner (p < 0.01) cortical neuronal NE, although the female hypertensive rats also showed a trend in the same direction. Nonneuronal levels of DA, NE, and EPI were similar to those of Wistar normotensive rats, with the exception of EPI in the male hypertensive animals, which in six of nine rats was up to 10 times greater than the range seen in the normotensive rats.

Effects of Salt Loading

Long-term salt loading had no appreciable effect on nonneuronal levels of any of the catecholamines in any renal zone, for either Sprague-Dawley or Wistar rats. The effects of salt loading on neuronal NE and DA are shown in Figure 2. Male and female rats of both normotensive strains showed a pronounced reduction in outer cortical neuronal NE, and an equivalent degree of reduction of inner cortical neuronal NE also was seen in the Sprague-Dawley strain. Although a qualitatively similar effect was seen in the inner cortex of Wistar rats, considerable intraindividual variability precluded this reaching significance. Salt loading had no consistent or pronounced effect on cortical DA in either normotensive strain.

The effects of salt loading on cortical neuronal catecholamines in the hypertensive Wistar strain were strikingly different from those produced in normotensive animals (see Figure 2). Neither cortical zone of the hypertensive animals exhibited any reduction of NE, while DA content was reduced significantly in the outer cortex of male and female rats, as well as in the inner cortex of male rats.

Discussion

According to the classification of Sternberg et al., the outer renal cortex contains proximal and distal convoluted tubules and glomeruli, while the inner cortex contains straight portions of proximal and distal tu-
Sprague-Dawley animals: compared with our results, comparisons to be made between our results and what two minor respects from our own observations on differentiated. The results obtained were different in stock. Neuronal and nonneuronal sources were not sized that considerable differences can exist between various strains. As the rats of the Dahl line sized that considerable differences can exist between control and treated groups for any difference of means between control and treated groups for any one strain is indicated by the asterisks (*p < 0.05, **p < 0.01, Mann-Whitney U test).

Figure 2. Comparison of neuronal dopamine (DA) and noradrenaline (NE) content in outer cortex (OC) and inner cortex (IC) of male (M) and female (F) rats, before and after salt loading. Control data for the three strains are denoted by open columns (Sprague-Dawley), diagonal hatching (Wistar Otago normotensive), and cross-hatching (Wistar genetically hypertensive). For each strain, the data from salt-loaded animals are shown as stippled columns. Results are expressed as means ± SEM. The population numbers for control animals are as in Figure I and for the salt-treated groups are 10 male, 12 female Sprague-Dawley rats; 10 male, 9 female normotensive Wistar rats; 10 male, 5 female hypertensive Wistar rats. Significance of difference of means between control and treated groups for any one strain is indicated by the asterisks (*p < 0.05, **p < 0.01, Mann-Whitney U test).

have been selectively bred for differential responses to sodium loading, they cannot be considered to be necessarily equivalent to random-bred rats of the same original line.

Comparison of catecholamine contents in an intact and a contralateral denervated kidney from the same rat enabled us to derive data for neuronal and nonneuronal contributions to total catecholamine content in each zone of the kidney. We found that virtually all NE in both outer and inner cortex, and in the outer part of the medulla, was neuronal in origin. A substantial proportion of outer cortical NE must represent vasomotor axons terminating on the juxtaglomerular microvasculature and innervating renin-secreting juxtaglomerular cells. However, there is also morphological and functional evidence for the direct sympathetic innervation of proximal and distal tubules. Although each of these types of NE-containing nerve enters the kidney at the hilus and thus travels through all renal zones, the negligible amount of neuronal NE detected in inner medulla suggests that the nonterminal nerves do not contribute substantially to the tissue concentrations measured. The distribution of neuronal NE seen is therefore likely to reflect the distribution of functional terminal nerves.

All noradrenergic neurons contain small amounts of DA as well as NE. Although the relative amounts of DA and NE vary considerably between species, within any one species the ratio is fairly constant from tissue to tissue. Previous studies using Sprague-Dawley rats have shown a DA:NE ratio for noradrenergic terminal nerves of about 1:25, which is closely similar to the values obtained in the present study; however, consistently lower ratios, approximating 1:50, were found in the renal nerves of the Wistar animals. This finding may reflect a strain difference in the balance between tyrosine hydroxylation and DA hydroxylation as rate-limiting steps in NE synthesis. There is abundant morphological, biochemical, and immunohistochemical evidence for the existence of dopaminergic sympathetic neurons in the renal nerve supply of the dog. Some functional data also suggest the involvement of dopaminergic nerves in regulation of renal function in the rat; however, the amounts of DA found in the present study were compatible with its presence entirely in noradrenergic neurons. While these data do not preclude the additional existence of dopaminergic renal nerves, they do indicate that any population is likely to be small relative to that in the dog kidney.

In addition to the substantial proportion of renal NE and DA localized to nerves, we found that all zones of the kidney contained some nonneuronal catecholamines. One source of nonneuronal catecholamines in rat kidney has been suggested to be a population of small, fluorescent cells that lie in the medullary region, but these cells have been shown to contain serotonin rather than a catecholamine and therefore do not contribute to tissue catecholamine content. The concentration of NE and DA primarily in inner medulla is compatible with their presence in tubular filtrate. On the other hand, EPI was distributed evenly through cortex and medulla. All three catecholamines are extracted.
from renal arterial blood and excreted, but EPI appears to be metabolized during its passage through the tubules to a considerably greater extent than are NE and DA. 19,20 This may account for the differences in zonal distributions that we observed. We found that long-term salt loading of normotensive rats substantially reduced neuronal NE stores in the renal cortex. Administration of high salt diets has been reported to cause variable changes in plasma and urinary catecholamine levels, 21 possibly reflecting different alterations of sympathetic drive to various effectors. However, it is well documented that the increased extracellular fluid volume caused by long-term salt loading activates low pressure atrial baroreceptors and produces a reflex decrease in renal sympathetic nerve activity and increased glomerular filtration. 22,23 Although under some circumstances changes in neuronal activity may alter turnover of a neurotransmitter without affecting its storage, 24 chronic alteration in sympathetic drive has been shown to affect both turnover and storage of NE; reduced activity lowers, and increased activity raises, neuronal NE content. 25-28 The lowered NE levels found in the kidney after salt loading are therefore likely to be secondary to decreased renal vasomotor tone.

In contrast to its effect in normotensive rats, we found that long-term volume loading did not reduce NE storage in cortical sympathetic nerves of Otago hypertensive animals. This result is compatible with the view that the hypertensive animals may be unable to reduce their renal sympathetic nerve activity appropriately in the face of a chronically increased blood volume. The same strain has previously been reported to be less efficient than genetically related normotensive animals in excreting a salt load. 30 Selective renal denervation delays development of high blood pressure in both the Otago and the Kyoto (spontaneously hypertensive rat) strains of hypertensive rats 31-33 and the latter also have been shown to possess higher renal sympathetic drive than do normotensive Kyoto animals. 34,35 While normotensive Wistar-Kyoto rats respond to a salt load with decreased peripheral resistance and lowered plasma NE concentration, both peripheral resistance and circulating NE levels are elevated in salt-loaded spontaneously hypertensive rats. 36,37 Inappropriate elevation of peripheral resistance during salt loading has also been found in Dahl salt-sensitive rats, 38 as well as in patients with borderline essential hypertension. 39 An inability to reduce renal or general sympathetic vasoconstrictor drive in the face of elevated sodium intake or blood volume may thus be an important factor in the establishment of idiopathic hypertension in both humans and experimental animals.

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