Renal Modulation of Urinary Catecholamine Excretion During Volume Expansion in the Dog

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SUMMARY The role of the kidney in handling the biologically active, unconjugated endogenous catecholamines epinephrine (E), norepinephrine (NE), dopamine (DA), and the O-methylated metabolite of NE, normetanephrine (NM), was studied in the anesthetized dog before and after volume expansion with isotonic saline by measuring renal arterial delivery, urinary excretion rate, and renal venous return of these materials. Net turnover in the renal metabolic compartment was estimated by comparing arterial delivery to urinary excretion and renal venous return. The kidney extracted E and produced NE, DA, and NM before and after volume expansion. After volume expansion a significant decrease in the extraction of E, an increase in the production of DA, and no change in the production of NE or NM was seen. Clearance of catecholamines and NM through the kidneys appeared to increase with volume expansion. The clearance of DA exceeded the clearance of creatinine (Cr) after volume expansion, while the clearance of NM exceeded that of Cr before and after volume expansion, indicating that urinary DA and NM are derived, in part, from processes other than glomerular filtration. These observations suggest an important role for the kidney in the modulation of the excretion of catecholamines and metabolites. This role must be considered before the urinary excretion rate of N, E, DA, and NM can be related to generalized sympathetic function. The observed increases in renal DA production after saline infusion suggest a possible natriuretic function of intrarenal DA.

(Hypertension 2: 383-389, 1980)

KEY WORDS • dopamine • epinephrine • norepinephrine • normetanephrine

QUANTIFICATION of the urinary excretion rate of the endogenous catecholamines nor-epinephrine (NE), epinephrine (E), and dopamine (DA), and catecholamine metabolites such as normetanephrine (NM) has been used to evaluate sympathetic nervous system activity in experimental animals and man. It is not clear, however, what factors regulate renal metabolism, renal clearance, and urinary excretion of these substances. Recent studies from our laboratory have suggested that urinary NE may be derived, in part, from intrarenal sources in man. In dogs, other investigators have found that the kidney appears important in modulating the urinary excretion of catecholamines. Little is known concerning the renal clearance of normetanephrine (NM).

Our present study examines the role of the kidney in the metabolism and urinary clearance of NE, E, DA, and NM. Experiments were performed in anesthetized dogs before and after volume expansion with saline since this maneuver has been widely used in human subjects to evaluate sympathetic nervous system function and is known to increase urinary DA excretion. Net catecholamine production or extraction by the kidney was evaluated by quantifying arterial delivery, renal clearance, and urinary excretion of these substances. Recent studies from our laboratory have suggested that urinary NE may be derived, in part, from intrarenal sources in man.

METHODS

Surgical Procedure

Seven male mongrel dogs weighing 19.9 ± 0.7 kg (se) were maintained on Purina dog chow. The animals were anesthetized with intravenous pentobarbital (30 mg/kg) and maintained with supplemental doses as needed. All animals were intubated. Venous catheters of PE 260 tubing were placed in both femoral veins. The left femoral vein catheter was advanced to the right atrium and used for infusion of maintenance solutions of creatinine and p-aminohippurate (PAH) (Sigma Chemical Company). The right femoral vein catheter was advanced to the distal inferior vena cava and used for all other infusions. Bilateral femoral artery catheters of the same tubing...
were inserted. The left femoral arterial catheter was placed just distal to the renal arteries and used to record distal aortic blood pressure (BP) via a Statham P23Db pressure transducer with recording via a polygraph (Brush Instrument Company, Cleveland, Ohio). The right femoral catheter was advanced to the aortic bifurcation and used to sample aortic blood. A right carotid artery catheter was placed in the intrathoracic descending aorta to measure proximal blood pressure via a Statham P23Db pressure transducer and the Brush polygraph. A priming dose of 0.5 g PAH and 4.30 g of creatinine (Cr) dissolved in 30 ml normal saline was given intravenously. A maintenance infusion of 5 g PAH and 14 g Cr dissolved in 150 ml of normal saline was given via the left femoral vein at a rate of 0.2 ml/min.

The left kidney was exposed with a subcostal incision. The left ureter was catheterized with PE 160 tubing to record urine flow and collect urine samples from the left kidney. The spermatic vein was freed of connective tissue and cannulated with PE 160 tubing and advanced to the left renal vein. The renal artery was freed of connective tissue and, with care to avoid injury to renal nerves, an electromagnetic flow probe was placed on the proximal renal artery; renal blood flow was then recorded using a CME flow meter (Carolina Medical Electronics, Inc., King, North Carolina) and a Brush polygraph. After the operation had been completed, the animal was suspended by a sling to provide access to the kidney, and a 1-hour recovery period was begun.

**Experimental Protocol**

After the recovery period, urine and midpoint blood samples obtained from the aorta and renal vein were collected for two, 15-minute control periods for Cr, PAH, sodium (Na), potassium (K), and osmolality. Blood samples were collected in heparinized tubes and placed on ice for catecholamines at the beginning, midpoint, and end of each period. The integral of the plasma concentration curve was used for clearance calculations. The samples were centrifuged in a refrigerated centrifuge at 4°C for 15 minutes at 2000 rpm. The plasma samples for catecholamines and NM were immediately frozen with dry ice. The remaining samples were kept refrigerated prior to analysis. The urine samples for catecholamine and NM determinations were acidified with glacial acetic acid and immediately frozen for subsequent analysis. Creatinine and PAH were determined by an autoanalyzer technique.11 Na and K were determined by flame photometry (Corning Instrument Company). Osmolality was determined by freezing point depression using an Advanced Instrument Osmometer.

After the two control periods, volume expansion was induced with intravenous 0.9% saline to a total of 5% of body weight over 30 minutes. The urine volume was replaced after each 15-minute period with additional 0.9% saline. After the 30 minutes of infusion, all 15-minute urine collections were obtained together with blood samples at the midpoint of each period except for catecholamines, which were obtained at the beginning, middle, and end of each period. Urine flow was determined by volumetric collection of urine in calibrated centrifuge tubes. Hematocrit was determined from arterial blood from each period by the Wintrobe technique. After the end of the protocol, both renal flow probes were calibrated in vivo and both kidneys were removed, weighed, and frozen. Pulse, systolic (SBP), diastolic blood pressure (DBP), mean BP, and renal blood flow (RBF) were determined at the beginning, midpoint, and end of each period. The weighted mean was calculated using the trapezoid approximation and was used for clearance determinations. Renal plasma flow was calculated by (1–Hct) × (RBF). In one animal we were unable to calibrate the flow probes, and in this animal PAH extraction was used to estimate RBF.

The NE, E, and DA were quantified by their conversion to tritiated O-methyl metabolites using highly purified catechol-O-methyl-transferase (COMT) and tritiated S-adenosyl methionine, with a modification of our previous methods.12-14 The COMT was purified from fresh rat liver; 100 g of tissue was homogenized in 4 volumes of cold 0.25 M sucrose using a polytron homogenizer. The homogenate was centrifuged for 15 minutes at 18,000 rpm in a Sorval centrifuge, 0-4°C. The supernatant was filtered through gauze and then centrifuged for 1 hour using a Beckman 45 Ti rotor at 186,000 g. The supernatant was adjusted to pH 4.9 with 2 N acetic acid and stirred for 15 minutes. The precipitate was removed by centrifugation for 15 minutes at 18,000 rpm. The supernatant was readjusted to pH 4.9, and ammonium sulfate was added to give a final concentration of 1.2 M. After stirring for 20 minutes at 0-3°C, the ammonium sulfate precipitate was removed by centrifugation for 15

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**Abbreviations Used**

- **ACA**: aortic concentration of catecholamines
- **AV**: arteriovenous
- **Cr**: potassium clearance
- **COMT**: catechol-O-methyl-transferase
- **CM**: clearance of sodium
- **Cm**: osmolar clearance
- **Cr**: creatinine
- **DA**: dopamine
- **DBP**: diastolic blood pressure
- **DTE**: diethylocthenyl
- **EDTA**: ethylenediaminetetraacetic acid
- **GFR**: glomerular filtration rate
- **NE**: norepinephrine
- **NM**: normetanephrine
- **PAH**: p-amino-hippurate
- **PNMT**: phenylethanolamine-N-methyl-transferase
- **RBF**: renal blood flow
- **RPF**: renal plasma flow
- **SBP**: systolic blood pressure
- **TES**: tris methyl-2-aminoethanesulfonic acid
- **Uc**: urine concentration of catecholamines
- **V**: urine flow
minutes at 18,000 rpm. Ammonium sulfate was added again to the supernatant to yield a final concentration of 2.0 M. After stirring 20 minutes at 0-3°C, the precipitate was removed as before. The pellet was resuspended to approximately 20 ml with the following buffer: 0.1 M TES, pH 8.0, 5 mM EDTA, 20% glycerol, and 1 mM diethylerhtitol (DTE). The resuspended pellet was applied to a 5 x 85 cm Sephadex G-50 column, which had been previously equilibrated with 25 mM BIS-TRIS, pH 6.0, 1 mM EDTA, 20% glycerol, and 1 mM DTE. The active fractions were pooled and concentrated to a volume of 10 ml using an Amicon PM 10 filter. The concentrated preparation was applied to a 1 x 16 cm DEAE-Sephadex column which had been equilibrated with the BIS-TRIS buffer described above. The enzyme was eluted with 0.15 M NaCl. The active fractions were pooled and concentrated to a final volume of 25 ml using an Amicon filter as before.

The enzyme was aliquoted into plastic test tubes and frozen at -20°C. The COMT was completely devoid of l-aromatic amino acid decarboxylase activity. The O-methylated products were formed by incubation as previously described14, 15 for 60 minutes. They were then extracted from potassium borate at pH 10.0 into toluene-isoamyl alcohol (3.2, v/v) and evaporated to dryness under vacuum and then resuspended in 25 ml of 0.1 M formic acid and 2.0 M formic acid, which was then evaporated to dryness in a vacuum centrifuge. The O-methylated products and added cold carrier were separated by thin layer chromatography on silica gel plates developed in chloroform, methanol, ethylamine, ammonium hydroxide (80:18:3:3, v/v). Each spot was visualized under ultraviolet light and with 2% bisdiethylhexyl phosphoric acid in toluene.

The NM was extracted from 250 ml of plasma using 2.5 ml of isooamyl alcohol. After mixing and centrifugation, 2.2 ml was transferred to tubes containing 200 ml of 0.1 M formic acid and 2.0 ml of toluene. The samples were shaken and centrifuged, and the organic phase was discarded. The aqueous phase was evaporated to dryness under vacuum and then resuspended in 25 ml of 1 M HCl. The NM was then converted to tritiated metanephrine using partially purified bovine adrenal phenylethanolamine-N-methyl-transferase (PNMT) with previously reported incubation techniques.14-18 Metanephrine was then extracted and separated by the same techniques used to quantify NE, E, DA. Urine was diluted and assayed without the initial solvent extraction. Cross-contamination by other substrates for PNMT was less than 1%. Internal standards for each sample were included. Interassay variation was 5.2%, and the sensitivity of the assay was 10.2 pg for standards added after the initial solvent extraction step; recovery of NM for the initial solvent extraction step was 48%.

Statistical analysis was performed by the Student t test for paired data or by least-squares linear regression analysis. The 95% limits of probability were accepted as significant using a two-tailed test except for analysis of data for which previous studies have reported an increase in values with volume expansion, where a one-tailed test was applied.

### Results

Volume expansion produced a significant increase in urine flow rate (V), systolic blood pressure (SBP), clearance of sodium (CNa); clearance of potassium (CK) omsolar clearance (Ctoms), and the excretion of Na and K. A significant decrease in hematocrit was observed (tables 1 and 2).

<table>
<thead>
<tr>
<th>Measure</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine flow rate (ml/min)</td>
<td>0.24 ± 0.04</td>
<td>1.07 ± 0.28*</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>118.3 = 7.9</td>
<td>125.3 = 7.3</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>156.0 = 9.9</td>
<td>168.9 ± 8.6*</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>94.0 = 9.6</td>
<td>94.9 = 9.9</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>148.6 = 13.0</td>
<td>152.1 = 9.3</td>
</tr>
<tr>
<td>Renal blood flow (ml/min)</td>
<td>303.9 ± 28.6</td>
<td>322.1 ± 37.1</td>
</tr>
<tr>
<td>Renal resistance units (mm Hg/ml/min)</td>
<td>0.43 = 0.08</td>
<td>0.44 = 0.007</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>50.9 ± 2.2</td>
<td>41.1 = 1.8†</td>
</tr>
</tbody>
</table>

*p < 0.05
†p < 0.001

<table>
<thead>
<tr>
<th>Function</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ccr (ml/min)</td>
<td>33.7 ± 3.2</td>
<td>39.1 ± 4.5</td>
</tr>
<tr>
<td>Cr (ml/min)</td>
<td>102.7 = 12.8</td>
<td>109.1 = 15.5</td>
</tr>
<tr>
<td>K (ml/min)</td>
<td>0.091 = 0.022</td>
<td>1.004 = 0.265*</td>
</tr>
<tr>
<td>C5 (ml/min)</td>
<td>0.76 ± 0.08</td>
<td>1.75 = 3.07†</td>
</tr>
<tr>
<td>Na excretion (µEq/min)</td>
<td>14.11 ± 3.40</td>
<td>145.73 ± 40.11*</td>
</tr>
<tr>
<td>K excretion (µEq/min)</td>
<td>21.66 ± 3.25</td>
<td>35.00 = 3.22†</td>
</tr>
<tr>
<td>Osmolar excretion (mEq/min)</td>
<td>218.9 ± 23.2</td>
<td>516.3 ± 86.5†</td>
</tr>
</tbody>
</table>

*p < 0.05
†p < 0.01
The arterial and venous levels of E, NE, DA, and NM in the control and experimental periods are presented in figure 1. During the control period, renal venous E was less than arterial E \((p < 0.001)\). No arteriovenous (AV) difference was observed for NE, DA, or NM. Volume expansion resulted in a significant decrease in both arterial and venous levels of E. The arterial level decreased from a control value of 188.3 ± 35 (SE) pg/ml to 56.7 ± 21.9 \((p < 0.001)\). The renal venous level of E decreased from 77.8 ± 27.9 pg/ml to 36.8 ± 18.1 \((p < 0.01)\). In contrast, no significant changes were noted in NE, DA, or NM levels in the aortic or renal venous blood after saline infusion, and no significant AV differences were noted for DA, NE, or NM.

The amount of each substance filtered was assumed to be equal to the arterial concentration of the substance times the glomerular filtration rate \((GFR \times P_{CA})\). Using this formula, we found that the amount of E filtered fell significantly from 0.112 ± 0.023 to 0.035 ± 0.13 ng/min/g KW \((p < 0.002)\) with saline administration. However, filtered NE, DA, and NM did not change significantly. The urinary excretion of E, NE, DA, and NM before and after volume expansion are presented in figure 2. There was a significant decrease in the excretion of E after volume expansion from 0.084 ± 0.019 to 0.030 ± 0.011 ng/min/g KW \((p < 0.025)\). The excretion of NE did not change significantly. DA excretion increased significantly with volume expansion \((0.050 ± 0.012 ng/min/g KW to 0.078 ± 0.023 ng/min/g KW (p < 0.05). The NM excretion increased significantly with volume expansion from 1.252 ± 0.225 to 1.951 ± 0.352 ng/min/g KW \((p < 0.05)\).

There was a significant increase in the clearance of epinephrine \((C_E)\), norepinephrine \((C_{NE})\), dopamine \((C_D)\), and NM \((C_{NM})\) after isotonic volume expansion (fig. 3). The \(C_E\) rose from 0.453 ± 0.075 ml/min/g K to 0.652 ± 0.104 \((p < 0.05)\). The fractional clearance of E rose insignificantly from 74% ± 6.1% to 93.6% ± 12.7% \((p > 0.05)\). The clearance of NE increased from 0.438 ± 0.062 to 0.680 ± 0.108 ml/min/g KW \((p < 0.025)\), and the fractional clearance of NE rose insignificantly from 76.1% ± 12.4% to 96.3% ± 8.1% \((p > 0.05)\). The clearance of DA rose from a control clearance of 0.711 ± 0.214 to 1.092 ± 0.316 ml/min/g KW \((p < 0.05)\) with volume expansion. The fractional clearance of DA rose insignificantly from 126.5% ± 14.2% to 160.8% ± 20.3% \((p > 0.05)\). The clearance of NM increased significantly from 1.335 ± 0.308 to 2.055 ± 0.522 ml/min/g KW \((p < 0.05)\), and fractional clearance rose significantly from 235.8% ± 44.1% to 295.2% ± 39.2% \((p < 0.05)\). Further, the clearance of NM was significantly greater than that of Cr in the control period \((p < 0.05)\), and the clearance of NM and DA both exceeded the clearance of Cr after volume expansion \((p < 0.05)\). The clearance of NE and E was less than that of Cr in the control period \((p < 0.05)\) but did not differ statistically from Cr clearance after volume expansion. The net turnover of free CA in the renal metabolic compartment was determined by the following formula:

\[
\text{Net turnover of free CA} = \frac{\text{EX}}{\text{VENOUS}} - \frac{\text{ARTERIAL}}{\text{VENOUS}}
\]
net catecholamine turnover =

\[ \text{RPF} \times V_{CA} + V \times U_{CA} - \text{RPF} \times A_{CA} \]

where RPF = renal plasma flow; V = urine flow; and \( V_{CA}, A_{CA}, \) and \( U_{CA} \) represent renal venous, aortic, and urinary concentrations of catecholamines respectively. Positive values indicate a net production of catecholamine, and negative values indicate a net removal of catecholamine by the kidney. The net return is defined as the sum of venous return and the urine excretion. The net amount of catecholamines and NM produced or removed by the kidney was calculated using this equation (table 3). In the control period E was extracted by the kidney; after volume expansion, the extraction fell. Delivered E was significantly greater than the net return of E in both the control and experimental periods (\( p < 0.05 \)). In the control period, NE, DA, and NM were produced by the kidney (table 3); after volume expansion, the production of DA increased (\( p < 0.05 \)) but did not change for NE or NM. The net return of DA was significantly greater than the DA delivered in both control and volume expansion periods (\( p < 0.05 \)).

**Table 3. Calculated Renal Turnover of Catecholamines**

<table>
<thead>
<tr>
<th>Catecholamine</th>
<th>Net production or addition (ng/min/g KW)</th>
<th>Net extraction (ng/min/g KW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epinephrine (E)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>0.237 ± 0.060</td>
</tr>
<tr>
<td>Experimental</td>
<td>7</td>
<td>0.039 ± 0.013*</td>
</tr>
<tr>
<td>Norepinephrine (NE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>0.158 ± 0.228</td>
</tr>
<tr>
<td>Experimental</td>
<td>7</td>
<td>0.346 ± 0.149</td>
</tr>
<tr>
<td>Dopamine (DA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>0.101 ± 0.034</td>
</tr>
<tr>
<td>Experimental</td>
<td>5</td>
<td>0.159 ± 0.036*</td>
</tr>
<tr>
<td>Normetanephrine (NM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>1.019 ± 1.031</td>
</tr>
<tr>
<td>Experimental</td>
<td>7</td>
<td>1.409 ± 0.093</td>
</tr>
</tbody>
</table>

*\( p < 0.05 \).

**Discussion**

We have previously reported that in standing humans a significant portion of urinary NE is derived from processes other than glomerular filtration. In addition, we have observed in humans that chronic oral salt-loading paradoxically suppresses the urinary NE secretion rate to a lesser degree than forearm plasma venous NE concentration. These observations, as well as a vast literature related to the urinary excretion of catecholamines and their metabolites, mandate a clearer understanding of how the kidney handles these substances. In our present
The plasma concentration of NM exceeded that of NE, E, and DA. No AV difference was observed across the kidney. Plasma concentrations of NM were not influenced by volume expansion. The renal clearance of NM exceeded Cr clearance in both the control and volume expansion period. Net renal production of NM occurred in both periods and was not affected by volume expansion. We conclude that the intrarenal metabolism of NE and NM are closely linked and that the kidney is the site of synthesis of a significant portion of urinary NM.

In this study, the hemodynamic and renal function data in the control period and following volume expansion agree with previous reports. In addition, Faucheux et al. and Carrier et al. observed a prompt fall in the sum of the NE and E levels in plasma measured as total catecholamines after volume expansion. We have found E levels to be 41% of the NE levels in arterial blood during the control period, and therefore a fall in E levels alone could produce a significant change if total catecholamines were quantitated. Similarly, we observed a decrease in the excretion of E but no change in NE excretion with volume expansion. Faucheux et al. reported a fall in urinary excretion when both catecholamines were measured together. The extraction of E by the kidney seen in both periods of the present experiment confirms the observations of Unger et al. In addition, previous studies in the dog have not demonstrated a significant difference in arterial and renal venous NE concentrations with acute stress; our observations are in agreement with these studies.

Overy et al. using infused tritiated NE, reported that the fractional clearance of NE was 59.1% in anesthetized dogs. This compares favorably to the fractional clearance for NE of 76.1% that we found during the control period. These investigators did not examine the effects of isotonic saline infusion. We have previously shown a rise in NE clearance despite a fall in Cr clearance with upright posture in man, and others have noted that the clearance of NE and DA can exceed GFR. Using fluorometric methodology for E determinations, Jones and Blake were unable to detect E in the plasma of anesthetized dogs. After infusions of E to increase plasma E concentration, they observed that the fractional clearance of E was greater than 100%.

Our observations are supported by those of Faucheux et al., who reported that, in the dog, volume expansion with isotonic saline but not with albumin increased both urinary DA excretion and the fractional clearance of DA. Similarly, Alexander et al. found that isotonic saline infusion increased urinary DA excretion in man. Since we found that the fractional clearance of DA exceeded unity at the same time that net renal production of DA was observed, it is likely that the increase in urinary DA seen after saline infusion was not due simply to increased renal tubular secretion. Since DA is natriuretic, it is attractive to hypothesize that the DA synthetic system activated by saline is of physiologic importance in modulating the fractional clearance of Na.
In conclusion, despite the chemical similarity of NE, E, DA, and NM, the apparent renal clearances of these substances are distinctly different. Control clearance of NE and E is less than Cr clearance, while that of DA approximates, and that of NM exceeds, Cr clearance. Saline volume expansion increases either the absolute or fractional clearance of all of these substances. Net production was observed for NE, DA, and NM in the renal metabolic compartment, suggesting that a portion of these compounds excreted in the urine may result from intrarenal synthesis or metabolism of these materials. Saline infusion increases intrarenal production of DA. The urinary excretion rate of NE, E, DA, and NM is modulated by the arterial delivery of these substances to the kidney and by variations in apparent renal clearance.

Acknowledgments

We gratefully acknowledge the surgical assistance of Mary Ann Neel and the analytical assistance of Carol Robideau and Ronald Bosher. Dr. Naomi Fineberg provided statistical assistance, and Nina Kingery provided secretarial help.

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Hypertension. 1980;2:383-389
doi: 10.1161/01.HYP.2.4.383

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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