Renal Vascular Response to Salt Restriction in Normal Man

Evidence Against Adrenergic Mediation

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SUMMARY
The role of the sympathetic nervous system in the renal vasoconstriction induced by sodium restriction has been assessed in normal man in balance on a 10-mEq sodium intake. Renal blood flow was measured with radioxenon injected into the renal artery. Phentolamine infused into the renal artery at rates of up to 3 mg/min did not increase net renal perfusion or alter its intrarenal pattern. That dose of phentolamine was at least 30 times the threshold for significant blockade of the renal vascular effects of a large dose of epinephrine injected intra-arterially. The results suggest strongly that neither circulating catecholamines nor the sympathetic nerves play a central role in the renal vascular response to salt restriction in normal man.

Additional Indexing Words:
Renal blood flow Renal salt handling Radioxenon Renal sympathetic nerves Intrarenal blood flow distribution Alpha-adrenergic blockade Phentolamine

SODIUM RESTRICTION is followed by a reduction in plasma volume and renal perfusion in man. The pattern of intrarenal blood flow distribution in the salt-depleted individual is remarkably similar to that induced by catecholamines, sympathetic nerve stimulation, congestive heart failure, and plasma volume depletion in the experimental animal and man, which are also stimuli to net sodium reabsorption by the kidney. Because several lines of evidence have suggested a direct role of the sympathetic nervous system in renal sodium homeostasis, it has been implicated as well in the renal vascular response to salt restriction. In this study, this possibility has been examined directly in normal man by assessing the renal vascular effects of adrenergic blockade induced with phentolamine infused into the renal artery in subjects on a restricted salt intake. Because it was possible in this way to achieve renal concentrations of phentolamine in excess of 30 times the threshold for blockade, the absence of a significant increase in renal blood flow suggests strongly that the sympathetic nervous system is not involved in the renal vascular response to salt restriction.

Methods
The subjects were potential kidney transplant donors, requiring assessment by selective renal
arteriography. They ranged in age from 27 to 59 years and were free of cardiovascular and renal disease, with the exception of one potential donor with mild essential hypertension. A detailed inpatient evaluation included a complete history and physical examination, repeated urinalyses and quantitative urine cultures, electrocardiogram, chest film, intravenous pyelogram, and renal arteriogram. Renal function was assessed by multiple determinations of 24-hr creatinine clearance.

All subjects were admitted to a metabolic ward and placed on a diet which included a daily intake of 10 mEq of sodium, 100 mEq of potassium, and 2500 ml of water for at least 4 days prior to study, when sodium balance was achieved. A dietician monitored sodium intake, and daily 24-hr urine collections were made for the determination of sodium balance.

Percutaneous selective renal artery catheterization by the Seldinger method was carried out as described in detail previously. The methods were modified in this study to allow the continuous monitoring of arterial pressure during the intrarrenal infusion of the vasoactive drugs. A coaxial catheter system was employed, with an outer preformed catheter (O. D., 2.2 mm; I. D., 1.4 mm) used for arterial pressure monitoring and the periodic injection of xenon and contrast agent. A smaller polyethylene internal catheter (P. E. 10) was used for the continuous infusion of normal saline containing heparin at a concentration of 10 mg/liter into the renal artery. The infusions were maintained at 0.76 ml/min with a Harvard infusion pump, and when the vasoactive drugs were infused their concentration in the solution was adjusted so that the appropriate dose would be delivered at that rate of infusion. Blood pressure was measured with a Statham transducer and was recorded along with the electrocardiogram on an Electronics for Medicine recorder equipped with an oscilloscope to provide for continuous monitoring and maximum patient safety. The pressure measurement also insured that the catheter system did not produce a significant partial occlusion of the renal artery.

Studies were carried out in 33 subjects. In 12 subjects, replicate serial determinations of renal blood flow were carried out without the infusion of vasoactive drugs. In another 10 subjects, phentolamine was infused intra-arterially after a control blood flow study; in six of the 10, at two or more log-dose levels. In the other four, phentolamine was infused at a single dose level from 0.1 to 3.0 mg/min, and a 6-μg bolus of epinephrine was injected to assess the degree of alpha-adrenergic blockade. The effect of the same dose of epinephrine on intrarenal hemodynamics in the absence of phentolamine was assessed in six subjects, as reported previously.

The ability of the xenon washout method to detect an increase in renal blood flow was assessed in another five subjects, with the intrarrenal infusion of acetylcholine at 10 μg/min.

The drugs utilized were phentolamine mesylate (Regitine; Ciba), acetylcholine chloride (Merck), and epinephrine hydrochloride (Adrenalin chloride; Parke-Davis). All doses have been calculated as the salt of the solution.

The methods used for assessing intrarenal hemodynamics with the xenon washout method in man are modified slightly from those used in the dog, as described in detail elsewhere, as described in detail elsewhere.

In brief, 133Xe in saline solution was injected into the renal artery through the outer catheter, and its disappearance from the kidney was monitored by external counting. Mean renal blood flow was calculated from the initial slope. To make serial blood flow studies possible a modified compartmental analysis was carried out on the data; the two slowest components were approximated by a single value. For xenon, the optimal constant has been found to be the count rate 3 min after the peak. Flow rate in the most rapid flow component was calculated from the rate constant and the percentage of flow entering this compartment from the zero-time intercept. The serial studies were carried out at intervals of 5 to 15 min, when the count rate had generally fallen to less than 5% of the peak count. Repeat selective arteriograms were also carried out during the infusion of the vasodilator drug at the end of the third blood flow determination.

Written permission was obtained for the study from each subject after a detailed description of the procedures. The protocols have been approved by the Human Experimentation Committee of the Peter Bent Brigham Hospital.

Mean values are followed by the standard error of the mean as the index of dispersion. Tests of statistical significance between means reported in this study were calculated either by the Student t-test, or by paired data analysis where appropriate. The significance of individual changes was also assessed by comparison with the 95% confidence interval in the relevant control population.

Results

Mean renal blood flow in this group of salt-restricted subjects was 274 ± 4 (SEM) ml/100 g/min prior to infusion of vasoactive agents, significantly less than our normal value of 338 ± 7 ml/100 g/min for renal blood flow in subjects on an unrestricted salt intake (P < 0.01).

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Changes in renal perfusion induced by acetylcholine (10 μg/min) and phentolamine, 0.1(●), 0.3(○), 1.0(●), and 3.0(●) mg/min, infused into the renal artery. A series of replicate studies are shown on the left, and the area representing the 95% confidence limits on curve replication is indicated. Note that acetylcholine induced a significant increase in flow whereas phentolamine did not.

The efficacy of alpha-adrenergic blockade induced by phentolamine was assessed with epinephrine injected into the renal artery during phentolamine infusion. The effect of a 6-μg bolus of epinephrine on mean renal blood flow is shown with the 95% confidence limits. During the infusion of phentolamine at dose levels above 0.1 mg/min, no epinephrine response occurred. A phentolamine dose of 0.1 mg/min decreased the epinephrine response significantly but did not obliterate it. Therefore, the maximum dose of phentolamine utilized exceeds by at least 30 times the threshold for effective blockade of a very large dose of epinephrine.
Phentolamine infused into the renal artery in doses ranging from 0.1 mg/min to 3.0 mg/min failed to alter renal blood flow (fig. 1) in all but one subject. In figure 1 the shaded area represents the 95% confidence limits about flow replication in the serial determinations in normal salt-restricted subjects. The only subject to show an increase in flow which fell outside our confidence interval for replication was the patient with essential hypertension, in whom a significant flow increase occurred with the infusion of both 1.0 and 3.0 mg/min.

Epinephrine injected into the renal artery reduced mean renal blood flow to from 29 to 46% of control levels in six subjects (fig. 2). Epinephrine-induced vasoconstriction was unrecognizable in the subjects receiving phentolamine at doses of 0.3 to 3.0 mg/min. In the subject receiving phentolamine at 0.1 mg/min, renal blood flow fell by 92 ml/100 g/min after epinephrine, a reduction outside both the confidence interval for flow replication and for the effect of epinephrine. This dose, therefore, represents the threshold for the blocking action of phentolamine in this system, when a large dose of epinephrine is used. Because phentolamine is a competitive blocking agent, it seems likely that a lower threshold would have been demonstrated if a smaller test dose of epinephrine had been used.

The acetylcholine infusion resulted in a significant increase in renal perfusion in all five subjects, ranging from 170 to 320 ml/100 g/min (fig. 1).

The findings from compartmental analysis paralleled changes in mean blood flow in all groups as shown in table 1. Sodium restriction resulted in a statistically significant reduction in the percentage of renal blood flow in the rapid flow component (69.1 ± 4.2%) when compared to normal subjects on an unrestricted salt intake (74 ± 1.0%, P < 0.01), as in the previous study.3 Phentolamine failed to reverse this response, but acetylcholine increased mean flow, the percentage of flow in the rapid flow component, and the rapid component flow rate significantly (P < 0.01). None of the vasoactive drugs infused changed arterial blood pressure at the doses reported. Attempted to increase the phentolamine dose resulted in a gradual reduction in arterial pressure and were abandoned.

Discussion

The precise role of the sympathetic nervous system in renal sodium handling remains unclear despite decades of investigation. Unequivocal evidence from animal models has demonstrated that sympathetic activity to the kidney does result in sodium retention, and suggests that such activity contributes to sodium retention in response to the severe stress provided by hemorrhage or heart failure.4, 5, 7, 10–14 The distribution of neural catecholamine stores in the kidney has been clearly defined by modern fluorescent techniques. The nerve supply is confined to arterial structures, ending at the afferent arteriole, suggesting strongly that the effects of sympathetic activity are mediated by vascular responses.21, 22

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td><strong>Effect of Phentolamine on Intrarenal Hemodynamics</strong></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Component I (%)</th>
<th>Mean flow (ml/100 g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>10</td>
<td>69.1 ± 4.2</td>
</tr>
<tr>
<td><strong>Phentolamine:</strong></td>
<td>0.1 mg/min</td>
<td>4</td>
</tr>
<tr>
<td>0.3 mg/min</td>
<td>4</td>
<td>73.3 ± 5.1</td>
</tr>
<tr>
<td>1.0 mg/min</td>
<td>6</td>
<td>71.0 ± 3.6</td>
</tr>
<tr>
<td>3.0 mg/min</td>
<td>4</td>
<td>62.0 ± 10.4</td>
</tr>
<tr>
<td><strong>Acetylcholine</strong></td>
<td>5</td>
<td>84.8 ± 1.8*</td>
</tr>
</tbody>
</table>

*P < 0.01 for difference between control and experimental determination.
The pattern of perfusion in the kidney associated with the blood flow reduction induced by salt restriction is strikingly similar to that resulting from mild sympathetic activation in both the experimental animal and man. This similarity and the well-documented effects of sympathetic activity on renal sodium handling have resulted in the attractive hypothesis that such activity plays a role in normal renal sodium handling. The effects of guanethidine, an agent which prevents neural release of catecholamines, on sodium homeostasis in man are consistent with this hypothesis. Treatment with guanethidine resulted in a significant impairment of normal subjects' ability to retain sodium in response to a low salt diet. The systemic administration of such an agent, however, inevitably must result in significant effects on all structures innervated by sympathetic nerves so that its effects on sodium homeostasis cannot be attributed with assurance to a primary action on the nerves to the kidney.

The results of the present study do not support hypotheses which suggest that the sympathetics mediate the reduction in renal perfusion induced by salt restriction. Before a negative pharmacologic study can be interpreted, two criteria must be met: (1) the dose range utilized must be demonstrated as adequate to have blocked the response under study, and (2) the assay system used must be shown capable of monitoring the response. Both criteria have been met in this study. The largest dose of phentolamine used, 3.0 mg/min, was more than 30 times the threshold dose inducing a significant reduction in the response to a large dose of epinephrine. It is well known that there is generally considerably more difficulty in blocking the effects of local, neurally-released, than circulating catecholamines. It has been demonstrated for phentolamine that it has no direct effect on the renal vessels, and that it is an effective blocker of sympathetic neural activity, the dose required to produce an equivalent blockade of neural catecholamines being only 3 to 10 times the dose for circulating catecholamines. A very large dose of epinephrine was used to assess the efficacy of the blockade. It appears likely, therefore, that the largest doses of phentolamine utilized in this study were adequate to have achieved a considerable blockade of sympathetic activity if such were present. It is clear that the effects of circulating catecholamines were effectively blocked. The prompt and dramatic response of the renal vasculature to the infusion of acetylcholine fulfilled the second criterion, demonstrating clearly that the vessels could respond acutely to an appropriate locally-administered vasodilator, and that the xenon washout method was sufficiently sensitive to measure the resultant flow increases.

These observations support the earlier suggestion that in normal supine man there is minimal sympathetic activity to the kidney, and extends it to include normal man in whom moderate plasma volume depletion has been induced by salt restriction. The approach utilized should be useful in delineating the role of the sympathetic nerves to the kidney in a number of processes. The chance observation that phentolamine induced a significant increase in renal perfusion in a patient with essential hypertension requires further investigation, especially in view of the hypertension induced in the experimental animal by chronic splanchnic stimulation. While most of the available evidence suggests that a diffuse increase in sympathetic activity does not occur in patients with essential hypertension, regionally differentiated autonomic activity is well documented in animal models and must be assessed in this setting.

The mechanism by which sodium restriction induces renal vasoconstriction remains unclear. The well-documented relationship between salt balance and renin secretion suggests the possibility that a local increase in angiotensin concentration contributes to the vasoconstriction. Recent studies have shown, however, that angiotensin octapeptide is unlikely to be a specific intrarenal hormone because the rate-limiting step in the renin-angiotensin sequence is a converting enzyme which is primarily found in lung.
The possibility that other, as yet undefined, mediators are involved in the renal vascular response to salt restriction requires further investigation. The definition of the mechanism accounting for the reduction in renal perfusion and its redistribution within the normal kidney should provide insights into both the mechanism of salt retention in states characterized by edema and certain abnormalities of sodium homeostasis in patients with essential hypertension.33

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