Renal Vascular Responses to Angiotensin and Norepinephrine in Normal Man

EFFECT OF SODIUM INTAKE

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ABSTRACT

The effect of sodium intake on renal vascular responses to angiotensin and norepinephrine infused intra-arterially was assessed in normal man by xenon washout. In subjects on an unrestricted diet, the dose of angiotensin inducing a 50% reduction in mean blood flow (ED\textsubscript{50}) was 38.2 ± 12.6 ng/min, a hundredfold lower dose than that for norepinephrine. Sodium restriction reduced the sensitivity to angiotensin tenfold: the ED\textsubscript{50} rose to 378 ± 12 ng/min. This diet, conversely, potentiated responses to norepinephrine: the threshold dose fell from 37 ± 10 to 3.6 ± 1.9 ng/min. Thus, the reduction in sensitivity to angiotensin induced by sodium restriction was not a nonspecific effect on the vascular smooth muscle. Sodium restriction also reduced the variability in the vascular response to angiotensin, and a close correlation was found between urine sodium content and angiotensin responsiveness. The blood vessels of the normal human kidney are remarkably sensitive to angiotensin: the threshold dose is approximately 1 ng/min for intra-arterial infusion. Circulating angiotensin may be a mediator of renal vascular tone, but circulating catecholamines probably play a minimal role.

KEY WORDS xenon washout intrarenal flow distribution vascular reactivity circulating catecholamines renal blood flow

Sodium restriction results in a striking reduction in the vascular response to angiotensin in the rabbit (1), but, conversely, the response to norepinephrine appears to be potentiated (1). It has been reported that sodium intake does not influence the renal vascular response to catecholamines administered intravenously in man (2), but this route of administration is not ideal for assessing vascular reactivity (3, 4). Also, potentiation of catecholamine effects induced

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Methods

These studies were carried out in 37 normal potential kidney donors at the time of their selective renal arterial catheterization for arteriography. The subjects ranged in age from 21 to 64 years; each was given a careful inpatient evaluation with special emphasis on cardiovascular, renal, and adrenal status to ascertain his normality. Details of the clinical evaluation have already been described (6). Subjects were admitted to a metabolic unit where 16 of the 37 were placed on a carefully controlled diet consisting of 10 mEq/day of sodium, 100 mEq/day of potassium and 2,500 ml/day of water for 4–5 days prior to study. The remainder were allowed an unrestricted sodium intake, which was monitored by measuring sodium excretion in 24-hour urine collections and by a detailed daily dietary assessment.

The techniques for the selective renal arterial catheterization, for the determination of renal blood flow with radioactive xenon and external probe counting, and for the administration of the vasoactive agents into the renal artery have been described in detail (6, 7). In brief, percutaneous selective renal arterial catheterization was achieved with local anesthesia under fluoroscopic guidance. A coaxial catheter system was employed (7): the inner catheter (PE 10) was used for continuously infusing heparinized saline or vasoactive agents and the outer catheter (red Kifa) for monitoring arterial blood pressure and injecting radioactive xenon. After a control determination of renal blood flow, either angiotensin amide (Hypertensin, Ciba) or norepinephrine bitartrate (Levophed, Winthrop) was infused into the renal artery in log-dose increments. Sixteen subjects, 7 of whom were on the low-sodium diet, received a norepinephrine infusion, and 21 subjects, 9 of whom were on the low-sodium diet, received an angiotensin infusion. The angiotensin doses ranged from 1 to 300 ng/min, and the norepinephrine doses ranged from 10 to 3,000 ng/min. The dose of both agents was calculated as the concentration of base. Fresh solutions were prepared by dilution in 0.9% sodium chloride just prior to each study. The concentrations were adjusted so that the infusion would deliver the appropriate dose at pump flow rates of 0.6–1.9 ml/min. Infusion at each dose level was carried out for 3 minutes before blood flow was determined. Four determinations were made at different dose levels in each subject, and the response to each dose level reported was assessed in at least 4 subjects. Mean blood flow was determined graphically from the initial slope, and the intrarenal distribution of blood flow was assessed by compartmental analysis. About 20% of the curves were selected at random for a computer assessment in addition to the graphical analysis.

Group means are presented with the standard error of the mean as the index of dispersion. Evaluation of statistical probability was carried out, where appropriate, with Student's t-test or the paired data t-test. Otherwise the Wilcoxon rank sum test for nonparametric data was used. The null hypothesis was rejected when the P value was less than 0.05.

The protocol was approved by the Human Experimentation Committee of the Peter Bent Brigham Hospital. Written permission for the procedure was obtained after a careful description of the protocols in every case.

Results

Angiotensin or norepinephrine infused into the renal artery did not induce a systemic hemodynamic response or a subjective response in any subject: mean arterial blood pressure, pulse pressure, pulse contour, and heart rate remained stable. The control values for renal blood flow and its intrarenal distribution were normal in all subjects, with characteristics similar to those described earlier (6, 7). The control mean blood flow ranged from 249 to 508 ml/100 g min⁻¹.
The threshold dose for norepinephrine was between 30 and 100 ng/min (Fig. 2). Increasing doses resulted in responses similar to those induced by angiotensin: progressively larger reductions in the mean blood flow, the percent of total renal blood flow in the rapid component, and the rapid-component flow rate occurred. The largest dose, 3 μg/min, resulted in the absence of a rapid flow component in one of five subjects.

A more rigorous definition of the threshold dose for each agent was obtained from the dose-response curve for change in blood flow in individual subjects. Each dose-response curve was fitted with a straight line selected on the basis of an unweighted least-squares solution. The threshold dose was derived from the abscissa intercept of that line. The ED₅₀, the dose reducing blood flow to 50% of control, was also defined from the fitted line, but only by interpolation for curves in which a 50% reduction in flow occurred. The threshold dose for angiotensin defined in this way was 0.5 ± 0.19 ng/min, with an ED₅₀ of 38.2 ± 12.8 ng/min. The threshold dose for norepinephrine was 37 ± 10 ng/min, approximately seventyfold higher on a weight basis than that for angiotensin (P < 0.02). Similarly, the ED₅₀ was 393 ng/min for norepinephrine, over a hundredfold higher than that for angiotensin (P < 0.01).

Sodium restriction had an opposite effect on the responses to the two agonists (Fig. 3). The threshold dose for angiotensin was increased about an order of magnitude from 0.5 ± 0.19 to 5.2 ± 1.15 ng/min (P < 0.005). Increasing doses resulted in increasing responses approximately parallel to those in subjects on an unrestricted diet: thus the ED₅₀ was also shifted tenfold from 38.2 ± 12.6 to 378 ± 12 ng/min (P < 0.005). The effects of sodium restriction on the responses to norepinephrine were more complex. The threshold dose fell significantly from 37 ± 10 to 3.6 ± 1.9 ng/min (P < 0.02). Responses to 30 ng/min were significantly smaller in subjects on an unrestricted diet. At this dose, the four subjects on an unrestricted diet showed
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Effect of sodium restriction on the renal vascular response to norepinephrine and angiotensin. Note the striking reduction in sensitivity to angiotensin induced by sodium restriction. The responses to norepinephrine are potentiated, primarily at threshold, by sodium restriction.

essentially no net change (4.0 ± 14 ml/100 g min⁻¹). Conversely, blood flow fell in each of seven subjects on the sodium-restricted regimen, with a mean reduction of 57 ± 7 ml/100 g min⁻¹ (t = 3.52, P < 0.01). With increasing doses, however, the responses to norepinephrine in subjects on the low-sodium diet were not significantly larger than those in the unrestricted group, and the dose-response curves merged, as is evident in Figure 3. The effects of the two vasoconstrictor agents on compartmental distribution of blood flow in the sodium-restricted group were essentially identical to those in the unrestricted group: there were progressive reductions in the percent of total renal blood flow in the rapid component and in the rapid-component flow rate.

Sodium restriction was also associated with a reduction in the variability of responsiveness to angiotensin. The average coefficient of variation of the responses to angiotensin in sodium-restricted subjects was 17.9 ± 6.9%, significantly less than that in the unrestricted subjects (44.7 ± 7.3%, P < 0.05). Spontaneous sodium intake in the unrestricted subjects ranged from 75 to 210 mEq/day, as assessed by urine sodium content and corroborated by dietary history. Urine sodium excretion was 3-42 mEq/day in the subjects on the restricted diet (Fig. 4). An excellent correlation was found between sodium excretion and the response to angiotensin at about the ED₅₀ dose level, 30 ng/min, as is evident in Figure 4 (n = 18, r = 0.70, P < 0.01). It appears, therefore, that the variability in response to angiotensin in the unrestricted population was accounted for, in large part, by spontaneous differences in sodium intake.

Computer analysis of randomly selected data showed an excellent correlation (r = 0.97) with the results from graphical analysis, as shown in Figure 5.

Discussion
Both angiotensin and norepinephrine induced a dose-related reduction in renal blood flow when they were infused into the renal
artery. This observation was not surprising: they are the two most active endogenous renal vasoconstrictor substances. Details of the dose-response relationship for angiotensin, however, were surprising. The threshold dose in normal subjects on an unrestricted sodium intake was less than 1 ng/min. With a normal blood flow and, thus, a renal plasma flow of approximately 300 ml/min, such an infusion would result in an increase in the inflow concentration of angiotensin of only about 3 pg/ml. Several recent studies with radioimmunoassay have shown that the normal arterial concentration of angiotensin is 20 pg/ml or more (8–10). The data available on the sensitivity of other vascular beds, for example the extremities in man, have revealed a considerably lower sensitivity (11). Although angiotensin is probably not present in arterial blood at levels sufficient to play an important role in maintaining vascular tone in somatic structures, the present observations imply that circulating angiotensin could be an important determinant of renal vascular tone. Furthermore, a number of recent observations have suggested that sufficient converting enzyme is present in the kidney to release locally the active moiety, angiotensin II, with renin release (12–14). It is likely, therefore, that intrarenal concentrations of angiotensin II are considerably greater than the concentration in arterial plasma. The present data are clearly consonant with a role for angiotensin in renal vascular homeostasis, especially in the control of intrarenal blood flow distribution.

The sensitivity of the renal vascular bed to norepinephrine, on the other hand, was much less striking; 30–100 ng/min were required in the normal subjects on an unrestricted diet for a threshold response, with an average value from regression of 37 ng/min. This dose resulted in a calculated increase in the inflow concentration of 0.1–0.3 ng/ml, an amount which approximates the normal level of the catecholamines in plasma (15). Thus, small changes in circulating catecholamines probably do not play a quantitatively important role as a determinant of renal vascular tone, although they may be important in the patient with pheochromocytoma or in severe shock. Neurally released catecholamines must be much more critical. These results are in accord with the elegant studies on the relative effects of neurally released and circulating catecholamines on vascular effector systems reported by Celander (16).
Most investigators have reported a pressor potency ratio of angiotensin to norepinephrine of approximately 10 to 1 (17–19). Similarly, a potency ratio of approximately 10 to 1 was defined in our earlier study on the vascular bed of the limb in the rabbit (1). The ratio of about 100 to 1 defined for the renal vascular bed in this study is unusual, reflecting the bed’s exquisite sensitivity to angiotensin. The kidneys receive 20% of the cardiac output, and skeletal muscle and skin also receive a considerable fraction; thus the overall 10 to 1 pressor ratio suggests that none of the other major vascular beds can approach the renal vasculature in its sensitivity to angiotensin. If another major vascular bed was this sensitive, the pressor response ratio would exceed 10 to 1. Earlier observations in man have shown that the renal vasculature indeed displays the greatest sensitivity to angiotensin (17, 19, 20).

The relative insensitivity to angiotensin of the renal vessels apparent in many animal studies (21–23) in which the kidney was exposed and manipulated may well reflect partial desensitization induced by the procedure. These studies have largely been carried out in the dog, in which specific tachyphylaxis of the renal vascular bed to angiotensin has been well documented (23). It would be of interest to explore renal vascular responsiveness to angiotensin in the unanesthetized animal.

The pattern of the renal vascular response to angiotensin and norepinephrine shown in this paper differed somewhat from that reported for both agents in the dog (5, 22). A reduction in mean renal blood flow was associated with a decrease in both the percent of blood flow to the rapid component and the rapid-component flow rate in this study. A reduction in the rapid-component flow rate was not reported in the canine studies. Whether this reflects a species difference, the effects of the anesthesia used in the studies in the dog, or the characteristics of the analytical technique is not clear. The significance of the rapid component defined by compartmental analysis has been reviewed in detail (8). It probably reflects cortical flow.

Sodium restriction induced striking and opposite changes in the responsiveness of the renal vasculature to angiotensin and norepinephrine: an order-of-magnitude reduction in sensitivity to angiotensin occurred, but the change in sensitivity to norepinephrine was much smaller and directionally opposite. The threshold dose for norepinephrine fell, whereas the responses to larger doses were essentially similar in unrestricted and restricted subjects. These effects are qualitatively similar to the effects of sodium restriction on the responses of the rabbit limb vessels. In the rabbit, a reduction in sensitivity to angiotensin occurred; however, the magnitude of the shift was considerably smaller. In the rabbit, the increase in sensitivity to norepinephrine induced by sodium restriction was also directionally similar to that in man, but the effect was considerably larger. In neither case did sodium restriction induce a parallel shift in the responsiveness to norepinephrine. The dominant effect was at the threshold level. Perhaps the fact that only the threshold response was influenced by sodium restriction accounts for the failure of earlier investigators to document an influence of sodium restriction on renal vascular responsiveness to norepinephrine in man (2). Kilcoyne and Cannon (5), however, did find norepinephrine potentiation in the renal vasculature of the sodium-restricted dog.

Possible mechanisms for the effects of sodium restriction in the rabbit on the actions of both agonists have been discussed in detail (1). Of significance here is the fact that the observations are clearly applicable to man and, thus, are not a peculiarity of the vascular responses of the rabbit. The much larger effects of sodium restriction on angiotensin responsiveness of the renal vasculature may shed some light on the mechanism. It would not be surprising if the largest increase in free angiotensin concentration induced by sodium restriction was within the kidney. Sodium restriction induces only a small increase in the free angiotensin level in arterial plasma (8–10). Recent observations have provided clear documentation of the presence of converting enzyme in the kidney (14) and of a remarkably high angiotensin concentration in
renal lymph (13). It is difficult to believe that a modest increase in plasma concentration induced a tenfold shift in sensitivity of the vascular bed to the agent. The studies cited above suggest that the increase in angiotensin concentration was considerably larger in the kidney. Thus, the largest decrease in sensitivity to angiotensin occurred at the site of the largest increase in angiotensin concentration. As pointed out in the earlier study, the loss of sensitivity to angiotensin induced by sodium restriction does not appear to be related to angiotensin tachyphylaxis (1). Tachyphylaxis to angiotensin does occur in the renal vascular bed of the dog (23), but available evidence reported in man suggests a well-sustained renal vascular response to smaller doses infused intravenously (17). The magnitude of the shift in sensitivity of the renal vessels to angiotensin induced by sodium restriction focuses attention on the kidney in the genesis of the desensitization.

In conclusion, the results of this study suggest that circulating and local angiotensin II levels play an important role in the control of renal vascular tone and blood flow distribution in man. Furthermore, the state of sodium balance must be considered in the assessment of renal vascular reactivity.

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