Renin release responses to acute alterations in renal arterial osmolarity

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RECENTLY WE HAVE SHOWN that renal blood flow (RBF) and glomerular filtration rate (GFR) change immediately in response to renal arterial infusions of small volumes of hypertonic solutions of NaCl, dextrose, or urea (19). The responses are believed to be the result of an osmotic action directly on the smooth muscle cells of the resistance vessels of the renal vascular bed. Increases in osmolarity probably cause an inhibition of contractile activity. The changes in renal hemodynamics produced by the infusions are undoubtedly accompanied by changes in the rate of sodium delivery to the macula densa region of the distal tubule. Hyperosmotic NaCl infusions especially would have dramatically increased both rate of delivery of Na to the macula densa and the Na concentration in the tubular fluid in the early distal tubule. According to the macula densa hypothesis (15), changes in rate of delivery of Na to the distal tubule or changes in its concentration there should affect renin release with increases in rate of delivery or concentration being associated with decreases in renin release. Nash et al. (8) provided the most direct evidence for the macula densa hypothesis by inhibiting the renin release that is stimulated by renal perfusion-pressure reduction or infusion of norepinephrine by infusing small volumes of concentrated NaCl solutions (0.76 ml/min of 1.8 or 3.5 M NaCl) into the renal arteries of anesthetized dogs. The present series of experiments was carried out to test further the validity of the macula densa hypothesis. Attempts were made to change the rate of delivery of Na to the distal tubule by altering renal functions by infusing solutions of hypertonic NaCl, dextrose, and urea into the renal arteries of anesthetized dogs while measuring changes in renin release. Renin release increased consistently in response to small acute elevations of renal arterial plasma osmolarity (RAPO) whether produced by infusion of solutions of NaCl, dextrose, or urea. The increases in release accompanied increases in RBF, GFR, and probably Na delivery to the macula densa region of the distal tubule. Rapid-sampling experiments revealed, however, that the increase was well established within considerably less than 15 sec of the start of hypertonic infusions. This demonstration of sharp increases in renin release while RBF, GFR, arterial sodium concentration, filtered sodium load, and undoubtedly sodium delivery to the macula densa were all elevated raises questions concerning the existence of an intratubular feedback mechanism which inhibits renin release in response to increases in distal tubule Na load.

METHODS

The procedures employed in these studies were similar to those described previously (19). Briefly, male mongrel dogs were anesthetized with sodium pentobarbital and maintained on positive-pressure respiration. The left kidney was approached retroperitoneally, the renal vessels were exposed and cannulated. A double-lumen cannula was placed in a femoral artery through which blood samples were drawn and pressure was measured. RBF was measured by a square-wave electromagnetic flowmeter (Carolina Medical Electronics, Inc.), GFR was determined from the product of the creatinine extraction ratio and the renal plasma flow. Urine flow rate was monitored by a photoelectric drop counter.

After completion of the surgery, a priming dose of creatinine was given followed by continuous infusion at a rate sufficient to maintain a stable plasma concentration between 10 and 15 mg/100 ml. Heparin was given 10 min before the first blood samples were taken. A 0.15 M NaCl infusion was begun into the renal artery at a rate of 0.8 ml/min.

Two 50 ml syringes in a double Harvard infusion pump were connected through three-way stopcocks and a Y leading to the renal artery cannula. Since both syringes permitted the same infusion rate but contained different
solutions, infusion of a different solution could be started without changing the infusion rate simply by turning the two stopcocks simultaneously. The exact time required for the new solution to travel from the syringe to the renal artery was determined for each infusion rate used.

Approximately 45 min after completion of the surgery, when arterial pressure, renal blood flow, and urine flow had stabilized, the experiment was begun. Simultaneously, 6 ml samples were withdrawn from the femoral artery and renal vein and placed in iced test tubes. Approximately 30 sec were required to draw the samples. Immediately after drawing these control samples, the syringe in the infusion pump containing 0.15 M NaCl was turned out of the system and the syringe containing the “experimental” hypertonic test solution was turned into the system. Infusion of the test solution was always at the same rate as the infusion during the control period. After the renal blood flow had stabilized at a new level, simultaneous arterial and venous samples were again drawn. The second samples were drawn approximately 2 min after the first pair, 30-60 sec after the start of hypertonic infusion. The test solution was then turned off and the 0.15 M NaCl infusion was resumed, again at the control rate. When renal blood flow and urine flow had stabilized at the control level, usually after 10-20 min, the next control samples were drawn and the procedure was repeated using another test solution.

The experimental solutions infused were 2.0 M NaCl, 4.0 M dextrose, and 4.0 M urea. Six dogs received infusions of NaCl; five dogs, dextrose; and five dogs, urea. The infusion rate was 0.8 ml/min, both during the control period during which 0.15 M NaCl was infused and during the experimental period when hypertonic solutions were infused.

To follow more accurately the time course of the renin release following hypertonic infusions, four rapid-sampling experiments were conducted in two dogs. Using a finger pump, samples were drawn at 15-sec intervals from the renal vein starting in the control period and continuing during the hypertonic infusion.

A Technicon AutoAnalyzer system was used to determine plasma concentration of sodium, potassium, and creatinine. Osmolarity of solutions and plasma was determined by an Advanced Instruments freezing-point-depression osmometer. Osmolarity changes were calculated as described previously (19) from the rate of infusion, the concentration of the infusate, and the renal plasma flow.

Renin concentrations in plasma samples were determined with the use of the method developed by Schrader (10). Of a plasma sample, 0.25 ml was added to 0.75 ml of partially purified dog-renin substrate (substrate concentrations equivalent to 800–1,000 μg angiotensin); the mixture was then placed in dialysis against 0.01 M EDTA at 4 C for 6 hr. After removal of calcium by the EDTA, the pH of the mixture was lowered by 18 hr of dialysis against a pH 5.3 phosphate buffer at 4 C. After dialysis, a small volume (0.025 ml) of saturated NaCl solution was added along with 0.025 ml of 40 mg/ml diisopropyl fluorophosphate. The mixture was then incubated in Siliclad-coated test tubes at 37 C for from 330 to 450 min. The reaction was stopped by heating to 95 C for 10 min. The angiotensin generated was extracted into phosphate buffer of pH 8.3, which increased the volume of the sample to 2 ml. The sample was thoroughly mixed, then centrifuged, and the supernate was stored frozen in Siliclad-coated test tubes for bioassay.

The angiotensin concentration in the sample was determined by the rat bioassay developed by Wathen et al. (17). Dibenzyline-, Ansolysen-treated, nephrectomized rats (250–300 g) were used. The angiotensin standard used was the synthetic angiotensin, Hypertensin-CIBA.

The renin concentrations in the original plasma samples were determined on the assumption that the renin reaction followed zero-order (substrate-independent) kinetics (10):

\[ \text{renin} = \frac{RPF \times (V_{\text{renin}} - A_{\text{renin}})}{KW} \]

where renin was expressed in Goldblatt units (GU) per milliliter, angiotensin was the angiotensin concentration in nanograms per milliliter of sample assayed, \( K_0 \) was the zero-order rate constant of the semi-purified substrate used, and \( T \) was the time of incubation in minutes. Renin release from the kidney, expressed in GU \( \times 10^{-4} \) per minute per gram kidney weight, was obtained from the product of the venous-arterial renin difference and renal plasma flow divided by the kidney weight (KW):

\[ \text{renin release} = \frac{RPF 	imes (V_{\text{renin}} - A_{\text{renin}})}{KW} \]

RESULTS

The hypertonic infusions produced a mean increase in RAP0 of 44.6 ± 2.5 (SE) mOsm/liter. This resulted in a mean increase in RBF to 112.0 ± 1.6 of the control value, while GFR increased to a mean value 114.8 ± 4.9 of the control level. There were no differences in the effects of the three solutes on renal hemodynamics during the brief infusion periods except that the response to urea infusion was short-lived as described in previous work (19).

Table 1 presents data from the 16 experiments involving hypertonic infusions. Shown in the table are the osmolarity increase, the renin release during infusion of the solution named, the renin release during the preceding control period, and the difference between the two. Also presented are the means, standard errors, and paired t test statistic for each group and for all three groups combined. Figure 1 is a graphic presentation of changes in renin release resulting from hypertonic infusion. In 15 of 16 experiments, renin release increased in response to hypertonic infusions. The one dog (1/27) that responded to 2 M NaCl infusion by decreasing renin release had a very low level of release during both the control period and during the hypertonic infusion, and the difference between the control release and the experimental release was small, −0.53 GU \( \times 10^{-4} \) min per g.

The rather broad scatter of the renin release data seen in Fig. 1 is probably largely attributable to the variability of the condition of the experimental animals when they entered the laboratory. It was not possible for the investigators to chronically precondition the animals before the experiments. As a result, there were surely differences in the animals' overall health, their electrolyte and water balances, and consequently in their renal renin content (9). Therefore, some scattering of the renin release data both during the control periods and during acute stimulation of the release mechanism (Table 1) is understandable. However,
RENIN RELEASE AND RENAL ARTERIAL OSMOLARITY

TABLE I. Renin release during acute hypertonic infusions

<table>
<thead>
<tr>
<th>Date</th>
<th>Solution infused: 2 M NaCl</th>
<th>Control</th>
<th>Diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/26</td>
<td>36 mOsm/L</td>
<td>1.08</td>
<td>-0.24</td>
</tr>
<tr>
<td>1/27</td>
<td>46</td>
<td>0.30</td>
<td>0.83</td>
</tr>
<tr>
<td>1/28</td>
<td>46</td>
<td>0.19</td>
<td>-0.07</td>
</tr>
<tr>
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</tr>
<tr>
<td>5/31</td>
<td>50</td>
<td>7.02</td>
<td>1.36</td>
</tr>
</tbody>
</table>

Mean: 49.5 ±3.9, SE ±1.27, P < .05

Solution infused: 4 M dextrose

<table>
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<th>Mean ±SE</th>
<th>Diff</th>
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</thead>
<tbody>
<tr>
<td>2/3</td>
<td>39 ±4.0</td>
<td>1.40</td>
</tr>
<tr>
<td>2/4</td>
<td>38 ±4.2</td>
<td>0.06</td>
</tr>
<tr>
<td>2/9</td>
<td>56 ±6.28</td>
<td>3.81</td>
</tr>
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<td>2/10</td>
<td>49 ±0.58</td>
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<tr>
<td>2/17</td>
<td>34 ±7.73</td>
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</tbody>
</table>

Mean: 43.2 ±4.40, SE ±1.22, P < .05

Solution infused: 4 M urea

<table>
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<th>Mean ±SE</th>
<th>Diff</th>
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<tbody>
<tr>
<td>2/25</td>
<td>42 ±2.40</td>
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<tr>
<td>2/29</td>
<td>53 ±1.16</td>
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<tr>
<td>3/1</td>
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<td>3/3</td>
<td>37 ±6.00</td>
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</tr>
<tr>
<td>5/24</td>
<td>25 ±3.02</td>
<td>-0.26</td>
</tr>
</tbody>
</table>

Mean: 40.0 ±4.18, SE ±1.00, P < .10

Combined Mean: 44.6 ±2.5, SE ±0.50, P < .001

Listed are the solution infused, date of experiment, osmolarity increase, renin release during the hypertonic infusion, renin release during the preceding control period, and difference between the two. Paired t test statistics are given for group differences. Results are rates of renin release expressed in GU X 10^-4/min per g KW. * Three times greater than the mean.

Despite the scatter, it is clear from the data that renin release can be associated with hypertonic solutions of NaCl, dextrose, and urea infused into the renal artery.

One-way analysis of variance of the three groups indicates that there are no differences among them; there were no differences in the effects of equally hypertonic solutions of NaCl, dextrose, and urea on renin release in these experiments. This is apparent from the presentation of the data in Fig. 1. Although the probability figure generated by the t test for the data from the five urea experiments, P < .10, is too low to be called significant, inspection of the data indicates that with little doubt urea does stimulate renin release and this probability value is misleadingly low. The low probability value is primarily due to the large group variance which results from the unusually large increase in renin release observed in the experiment of 2/29 (see asterisk, Table 1). This increase, 0.09 GU X 10^-4/min per g, is nearly 3 times greater than the mean for the urea group and approximately 6 GU X 10^-4/min per g greater than any other value in the group. If this value is deleted from the analysis, the remaining four values have a mean considerably lower (x = 1.58 GU X 10^-4/min per g) but the t test probability value associated with this mean is P < .05. This increase in probability level results from the decrease in the variance of the group not containing the extremely high value.

The results of these renin release studies indicate that acute increases in plasma osmolarity resulting from infusion of NaCl, dextrose, or urea solutions stimulate renin release within 1 to 2 min of the start of infusion. More precise information concerning the time course of the release is necessary to be able to come to any conclusions concerning the role of the macula densa in this type of renin release, since from 30 to 60 sec, depending on the rate of diuresis, are required for filtrate to move from the glomerulus to the macula densa region of the distal tubule under normal conditions (F. G. Knox, personal communication).

Therefore, four rapid-sampling experiments were conducted in two dogs. Samples were drawn every 15 sec starting before infusion of the hypertonic solutions and continuing for 1 to 2 min after the infusions. Dextrose, 4 M, was infused in two experiments, 2 M NaCl and 4 M urea each in one experiment. In each of the rapid-sampling experiments renin release increased within the first sample period (15 sec) after the beginning of osmolarity increase. Figure 2 depicts the results of a rapid-sampling experiment involving 4 M urea infusion for 45 sec. One pair of continuous control samples was drawn from the renal vein and femoral artery for 5 min prior to the start of rapid sampling from the renal vein (from -300 to 0 sec). At t = 0, renal venous sampling at 15-sec intervals was begun. From t = 40 to t = 85, 4 M urea was infused into the renal artery at 0.8 ml/min. Prior to hypertonic infusion, renin release was near zero. In the first sample period following the start of hypertonic urea infusion, definite increases in renin release were evident. In the next sample period a large release was apparent and it continued for the duration of the infusion. The abruptness of the termination of the release when urea infusion ended was of special interest: in the last period during which urea was infused, renin release was 2.95 GU X 10^-4/min per g; 15 sec later the release was -0.37 GU X 10^-4/min per g.
The blood flow undershoot characteristic of the hypertonic samples were drawn continuously from renal vein starting at t = 0. Shown are responses of renin release, renal blood flow, glomerular filtration rate, renal venous plasma sodium, and potassium concentrations (V[Na] and V[K]). Time course of urea infusion is shown at bottom of figure.

The blood flow undershoot characteristic of the hypertonic urea off-response (19) coincided with the cessation of renin release. For the remaining duration of the experiment, RBF increased toward the control level, finally overshooting it. Renin release increased at the end of the experiment, paralleling changes in RBF.

Negative renin release values were occasionally observed in this study. Brown et al. (2) have reported reninlike activity in human urine. Apparently these results are not wholly artifactual but may represent uptake or excretion of renin by the kidney. However, small, negative release values could have resulted from error in the renin assay.

DISCUSSION

The results of these experiments indicate that acute increases in RAPO stimulate the release of renin from the kidney. Release increased in 15 of 16 experiments involving infusions of hypertonic NaCl, dextrose, and urea and rose to a mean value during the infusions more than threefold higher than the mean control level. No difference was apparent among the renin-releasing effects of the three solutes used to increase osmolarity. The rapid-sampling experiments demonstrated that the release began within considerably less than 15 sec of the start of the osmolarity increase. The increase in release coincided with marked increases in RBF and GFR and, therefore, also with elevations of filtered sodium load. In all cases, especially in the experiments involving infusions of 2 M NaCl, rate of Na delivery to the macula densa region of the distal tubule would have been elevated by the infusions. Acting as osmotic diuretics, dextrose and urea would have inhibited proximal sodium reabsorption (3, 18), thereby allowing an increased amount of sodium to enter the distal tubule (15). An NaCl infusion of 2 M could have saturated the proximal tubule sodium reabsorbptive mechanism permitting an increased rate of delivery of Na to the macula densa region of the distal tubule. Clearly, no inverse relation between renin release and macula densa sodium load existed in the present experiments. Unfortunately, the effects of the infusions on sodium concentration in the early distal tubule cannot be determined at the present time. However, it seems unlikely that the three solutes used produced the same effect on sodium concentration at the macula densa, whereas they did have a similar effects on renin release. Therefore, it also seems unlikely that in these experiments there was a correlation, positive or negative, between sodium concentration in the tubular fluid at the macula densa and renin release.

The rapidity of the renin release response to osmolarity increase seen in the rapid-sampling experiments rules out any involvement of an intratubular element located in the distal tubule in the control of renin release in this experimental model. The release was well established 15 sec after the start of infusions. In addition, the release was completely ended 15 sec after the return of RAPO to the control value after urea infusion (Fig. 2). The samples collected during the rapid-sampling experiments were drawn continuously by a finger pump, the collection tube being changed every 15 sec. Therefore, the renin concentrations obtained from these samples were true mean values for the 15-sec period. For the renin concentrations to have changed as sharply as they did in the rapid-sampling experiments, the renin release rate must have responded almost immediately to the alterations of RAPO. The intratubular environment at the level of the macula densa could not have been altered at the time the release mechanism responded.

Hypertonic infusions could have stimulated a release of renin within seconds only by acting on an intrarenal mechanism. There are several possible ways to account for this rapid stimulation. First, increases in RAPO could cause a release of norepinephrine from the sympathetic nerve endings in the kidney, the transmitter then stimulating the release (17). However, increases in osmolarity in the range studied in this experiment have not been reported to cause norepinephrine release from sympathetic nerves (7). Second, the increases in RAPO could have caused a redistribution of intrarenal blood flow, as does occur following administration of other vasodilator substances (6, 12). If underperfusion of the outer cortex resulted, renin release could have resulted from stimulation of the baroreceptor mechanism of the afferent arterioles or juxtaglomerular (JG) cells (14). This conclusion is not supported by the observations that other vasodilators including papaverine (5) and personal observation), acetylcholine (13), and hydralazine (16) do not stimulate renin release from kidneys perfused at normal pressures.

The third possible explanation of the immediate stimulation of renin release by hypertonic infusions is that the JG cells per se were directly stimulated to release renin by the acute increases in RAPO. This hypothesis receives strongest support from the fact that no other explanation involving known stimuli for renin release can adequately account for the results obtained in these experiments. The hypothesis receives indirect support from the work of others.

Johansson and Jonsson (4) and Arvill et al. (1) studied...
the effects of changes in osmolarity on vascular smooth muscle in vitro rat portal vein preparations. They demonstrated that the smooth muscle cells of the preparation behaved as osmometers in response to changes in bathing medium osmolarity; that is, increases in extracellular osmolarity caused the cells to decrease in volume. Furthermore, they observed a significant correlation between changes in cell volume and the mechanical and electrical activity of the cells, decreases in volume being associated with inhibition of mechanical and electrical activity. They postulated that changes in cell volume affect membrane ion permeabilities and that this, in turn, is the factor influencing electrical and mechanical activity. Evidence has been presented which suggests that the smooth muscle cells of the renal vascular bed behave in a manner similar to those studied in vitro by Johansson and Jonsson (4); increases in RAPO resulted in decreased resistance due to inhibition of the mechanical activity of the cells of the resistance vessels (19).

Using renal resistance changes as an index of changes in vascular cell volume, the data from the present experiments indicate that stimulation of the renin release mechanism of the JG cells coincides with decreases in cell volume. The coincidence is especially evident in the rapid-sampling experiments. Figure 2 illustrates the results of the rapid-sampling experiments during which 4 m urea was infused. Renin release increased immediately along with the probable decrease in cell volume (as indicated by the decrease in renal resistance). As discussed previously (19), cessation of hypertonic urea infusion can be expected to produce transient increases in cell volume and stimulation of vascular smooth muscle activity. At the time the urea infusion ended and cell volume was probably increasing, an incredibly abrupt termination of renin release occurred, going from 2.95 GU X 10^-4/min per g during the last period in which hypertonic urea was infused to 0.07 GU X 10^-4/min per g during the next sample period 15 sec later.

The JG cells of the afferent arterioles were exposed to the same osmolarity changes as the adjacent smooth muscle cells; there is no reason to believe their volume response was different from that of the smooth muscle cells. Therefore, at this time the data encourage speculation about the existence of a relationship between some aspect of decreases in JG cell volume and stimulation of the release of renin. Just what aspect of cell volume decrease is to be associated with release of renin is difficult to determine.

As was mentioned above, Arvill, Johansson, and Jonsson (1, 4) found that decreases in cell volume produced inhibition of electrical activity in their preparation, suggesting that the cell membranes were hyperpolarized. It is not impossible that hyperpolarization of the JG cell membranes is a stimulus for the release of renin. This speculation receives some support from the fact that renal perfusion-pressure reductions stimulate renin release and undoubtedly hyperpolarize the smooth muscle cell membranes of the renal vascular bed (11) and, possibly, hyperpolarize the membrane of the JG cells themselves. Unfortunately, this relation between JG cell membrane potential and renin release probably will remain speculative well into the future.

In summary, the data from the present experiments indicate that acute increases in RAPO stimulate renin release by an extratubular osmotic effect possibly directly on the JG cells themselves. The release begins within considerably less than 15 sec of the start of osmolarity increase and continues during the time that RBP, GFR, and sodium delivery to the distal tubule are elevated. The data do not support the existence of an intratubular sodium-sensing mechanism which affects the acute onset of the release of renin; however, the results of these acute experiments do not rule out a role for the macula densa as a regulator of the environment of the JG cells over a longer period of time.

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