SUMMARY. Renin release was measured in the isolated rat kidney perfused with a recirculating artificial medium containing bovine serum albumin at 6.7 g per 100 ml or 11 g per 100 ml. At the higher concentration of albumin, glomerular filtration ceased and the rate of renin release over 70 minutes of perfusion was increased 6-fold. The addition of ouabain to the perfusate containing 11 g per 100 ml inhibited the release of renin, suggesting that inhibition of Na-K ATPase or the related changes in cellular volume or composition prevented renin release. Lowering the osmolality of the perfusate by reducing the concentration of sodium chloride also prevented the increase in renin secretion produced by perfusion with 11 g per 100 ml albumin. Increasing the osmolality of the perfusate with mannitol restored the augmented renin release. These results are consistent with the hypothesis that alterations in the volume of certain cells, perhaps in the juxtaglomerular apparatus itself, can control renin release. (Circ Res 50: 400-404, 1982)

SECRETION of renin by the kidney is thought to be controlled by several different mechanisms. Two major influences include intrarenal arterial pressure mediated via a posited intrarenal baroreceptor and distal tubular salt transport via the macula densa (Vander, 1964; Davis, 1976). It is difficult to distinguish the separate effects of these factors in experiments using intact animals since they tend to change simultaneously with any single perturbation. For this reason, a variety of experimental preparations have been utilized to study renin secretion, including isolated perfused kidneys (Fray, 1976, 1977, 1978), slices of renal cortical tissue (Weinberger, 1972; Lyons, 1974; Churchill, 1979) suspensions of renal cortical cells (Lyons, 1975) or glomeruli (Frederiksen, 1975), and kidneys in which glomerular filtration has been reduced by ureteral ligation accompanied by a period of renal ischemia (Blaine, 1970; Shade, 1972; Freeman, 1974).

The present experiments were designed to minimize or eliminate the effects of glomerular filtration and tubular reabsorption on renin release in a way other than by ureteral occlusion. This was accomplished by perfusing the isolated rat kidney with a solution containing a high concentration of albumin to achieve an intravascular oncotic pressure exceeding the hydrostatic pressure present in glomerular capillaries. In these circumstances, glomerular filtration is effectively eliminated, but renal perfusion pressure and flow are unaltered (Johnson, 1977; Kau, 1977; Swartz, 1978; Maack, 1980). With suppression of glomerular filtration, delivery of solute to the macula densa and stimuli to the macula densa derived from the tubular fluid are eliminated. Release of renin in this circumstance would be controlled solely by the arteriolar baroreceptors. When this technique was utilized, it was discovered that solutions containing high concentrations of albumin markedly stimulated the secretion of renin by the isolated rat kidney.

Methods

Male Sprague-Dawley rats of the Charles River strain, weighing 350-420 g and maintained on Purina Rat Chow were used. The right kidney was isolated and perfused by the method of Ross et al. (1973). The perfusate was prepared from bovine serum albumin dialyzed against a buffered Krebs-Henseleit solution that contained the following electrolytes (mm): Na⁺, 145; K⁺, 4.0; Ca²⁺, 2.5; Mg²⁺, 1.2; Cl⁻, 103; HCO₃⁻, 25, at a pH of 7.40. Five mm glucose was added as the sole substrate. Final albumin concentration was either 6.7 per 100 ml in control experiments and 11.0 per 100 ml to prevent glomerular filtration. The concentrations of calcium and of magnesium in an ultrafiltrate of either perfusate were measured by atomic absorption spectrophotometry or calcium electrode (Orion) and found to be identical with the concentrations of those ions in the Krebs-Henseleit dialysate.

The medium was pumped via a peristaltic pump into a glass oxygenator where it equilibrated with a gaseous mixture of 95% O₂ and 5% CO₂ phase and was then pumped by a second peristaltic pump into the renal artery via a glass cannula. The venous effluent was allowed to drain from the kidney into a reservoir from which it was recirculated. Pressure was controlled by a needle valve placed in parallel to the arterial line and flow was measured using an in-line flowmeter. Pressure was held constant at a mean pressure of 110 mm Hg for all experiments. One-milliliter samples of perfusate were removed at the intervals indicated and frozen at −20°C prior to renin assay. The volume of perfusate for every collection period was calculated back from the final measured volume, the sampling volume, and the urine volume whenever urine was formed. Previous experience with this preparation has shown that, under the constant temperature conditions of the enclosure where the
perfusion is done, the rate of evaporative loss of water is 0.06 ml/min; therefore a constant infusion of distilled water at that rate is now routinely done. With this procedure, albumin and sodium concentrations remain constant during the experiment.

In experiments with ouabain, the glycoside was dissolved in 1 ml of boiling distilled water and added to the medium just prior to the perfusion to produce a concentration of $10^{-3}$ M in the perfusate.

A standard, commercially available renin assay (New England Nuclear) for angiotensin I (AI) was utilized. Since an artificial perfusate was used, the addition of dog substrate was required to generate angiotensin I (Fray, 1976); no angiotensin I could be demonstrated in perfusate samples before renin substrate was added. An equal volume of this renin substrate was added to the perfusate sample just prior to incubation. Angiotensinase inhibitors (dimercaprol and 8-hydroxyquinoline) were added prior to incubation with substrate. A 1:1 ratio of perfusate sample to dog substrate yields reproducible values for renin activity that are independent of further addition of substrate and linear with increasing renin activity. Since the artificial perfusate recirculates, renin activity measured at any time represents the cumulative activity. No appreciable amount of renin is lost through urinary excretion (Fray, 1976). For purposes of comparison, the rate of renin release was calculated as follows:

$$\text{RR} = \frac{\text{(angiotensin I units/ml)} \times V}{t} (\text{angiotensin I units/ml})_{n-1} \times V_{n-1}$$

where: RR = rate of renin release in angiotensin I units per minute, $n = \text{experimental sample}$, $n-1 = \text{previous experimental sample}$, angiotensin I units = ng of angiotensin I per ml of sample generated in 1 hour of incubation, $V = \text{total volume of perfusate}$, and $t = \text{time elapsed between experimental periods (in minutes)}$.

All samples were compared at the same time intervals using Student's t-test. Values are reported as mean ± SEM.

Results

Effect of Increasing Albumin Concentrations in the Per fusate on Renin Release in the Isolated Perfused Rat Kidney (Table 1)

The rate of renin release rose progressively with time in control filtering kidneys perfused with 6.7 g/100 ml albumin, reaching a level of 9.8 ± 2.3 AI units per minute during the period 50-70 minutes of perfusion. A similar increase in the rate of renin accumulation with time was observed by Fray in this model of the isolated perfused rat kidney (Fray, 1976, 1977, 1978). The average renin release rate over a 70-minute perfusion was 4.8 ± 0.96 AI units/min.

Glomerular filtration was prevented in isolated perfused rat kidneys by using an albumin concentration of 11 g per 100 ml in the perfusate. This concentration of albumin is equivalent to an oncotic pressure of 70 mm Hg, calculated using the Landis-Tappenberg equation (Landis, 1963), slightly more than double that of normal 6.7% albumin concentration, and higher than the average rat glomerular capillary hydrostatic pressure. That filtration ceased under these circumstances was documented by the observations that urine was no longer formed; no tubular phase was discerned after the intrarterial injection of the dye lissamine green; the concentration of $^{14}$C-inulin in the recirculating perfusate increased during the experiment in contrast to experiments with filtering kidneys in which it falls (Swartz, 1978); after perfusion with ferrocyanide the Prussian blue reaction was absent in the lumen of cortical tubules; and “snap-frozen” sections of the kidneys perfused with 11 g per 100 ml of albumin showed tubular collapse as compared with those perfused with 6.7 g per 100 ml and filtering normally.

Perfusion with 11 g per 100 ml albumin in 12 experiments greatly increased renin release throughout the perfusion and in all periods studied. The average rate of release over the entire 70 minutes was 28.33 ± 4.46 AI units per minute, representing more than a six-fold increase over that seen in the control kidneys perfused with a lower concentration of albumin, in which glomerular filtration was allowed to proceed. The rate of flow of perfusate was essentially unchanged (40.0 ± 1.6 ml/min in nonfiltering vs. 38.5 ± 3.6 ml/min in filtering controls) and the calculated renal vascular resistance was unaltered.

Effect of Ouabain on Renin Release

With $10^{-3}$ M ouabain, a concentration that completely inhibits Na-K-ATPase (Ross et al., 1973), in the perfusate of 10 non-filtering kidneys, the release of renin was almost completely inhibited. With ouabain, renin production by non-filtering kidneys fell even below the level seen in normal filtering kidneys (experiment 3). Perfusate flow and, hence, renal resistance were not significantly changed by ouabain.

Effect of Altering the Osmolality of the Perfusate on Renin Release in the Non-filtering Kidney

When non-filtering kidneys were perfused with a sodium concentration of 100 mm, renin release was considerably diminished, reaching only 10% of its previous value (experiment 4). This striking effect on renin release of lowering the salt content of the solution containing 11 g per 100 ml of bovine albumin while perfusing the non-filtering kidneys appeared to be due to changes in osmotic pressure rather than in sodium concentration. When non-filtering kidneys were perfused with a solution containing 100 mm sodium but in which osmolality was normal at 300 mOsm/kg because of the addition of 100 mm mannitol, renin release was found to be at the levels usually seen in the non-filtering kidney (experiment 5).

Discussion

The rate of renin release by the isolated perfused rat kidney increases linearly with time, a fact that has been previously shown in this preparation (Fray, 1976, 1977, 1978). The mechanism for this continued increase in the rate of renin release is not known, but we should like to speculate that it may be due to the
Table 1
Renin Release by Isolated Perfused Rat Kidneys

<table>
<thead>
<tr>
<th>Experiment</th>
<th>AI units/min</th>
<th>Perfusate flow (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-30 min</td>
<td>30-50 min</td>
</tr>
<tr>
<td>1. Filtering kidneys</td>
<td>0.8 ± 0.5</td>
<td>4.8 ± 1.5</td>
</tr>
<tr>
<td>Albumin 6.7 per 100 ml (n = 7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Non-filtering kidneys</td>
<td>6.97 ± 1.64</td>
<td>52.22 ± 9.17</td>
</tr>
<tr>
<td>Albumin 11 g per 100 ml</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sodium 140 mM (n = 12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Non-filtering kidneys</td>
<td>0.02 ± 0.01</td>
<td>0.39 ± 0.26</td>
</tr>
<tr>
<td>Albumin 11 g per 100 ml</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sodium 140 mM (n = 10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ouabain 10^{-3} M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Non-filtering kidneys</td>
<td>0.67 ± 0.22</td>
<td>1.69 ± 0.39</td>
</tr>
<tr>
<td>Albumin 11 g per 100 ml</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sodium 100 mM (n = 12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Non-filtering kidneys</td>
<td>8.87 ± 1.43</td>
<td>55.79 ± 10.01</td>
</tr>
<tr>
<td>Albumin 11 g per 100 ml</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Sodium 100 mM (n = 9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mannitol 100 mM</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

interruption of a suppressive feedback loop such as the lack of generation of angiotensin in the absence of renin substrate and/or the absence of other circulating agents that control renin release and are normally present in the blood but absent in the artificial solution with which the kidney is perfused. It is interesting in this regard that, in isolated kidneys perfused with an albumin concentration of 11 g per 100 ml, the rate of renin release rises linearly with time only during the first 50 minutes of perfusion decreasing to a lower rate after that as if the high initial rate of release reduces the intracellular stores and/or synthesis of renin.

The effect of a high concentration of albumin to stimulate renin release was completely eliminated by adding ouabain to the perfusing solution. Ouabain appears to inhibit renin release by intact dog kidneys (Churchill, 1976) and slices of rat kidneys (Lyons, 1974; Churchill, 1979) but not by rat renal cortical cell suspensions (Lyons, 1975). The inhibitory effect has been attributed to an increase in intracellular calcium hypothesized to occur via a Na-Ca exchange mechanism (Churchill, 1979). We wondered whether the action of ouabain in our experiments might be related to cellular swelling through the inhibition of Na-K-ATPase, thereby counteracting a possible effect of high albumin concentration to reduce the volume of kidney cells, especially those in the juxtaglomerular apparatus.

Accordingly, an attempt was made to inhibit renin release by reducing the osmotic pressure of the perfusate in the non-filtering kidney. This was accomplished by perfusing with a solution of an osmolality of 200 mOsm/liter lower than the normal 300 mOsm/L. The reduction in osmolality was achieved by reducing the sodium chloride concentration of the perfusate. Like ouabain, this solution would be expected to produce swelling of cells. The stimulation of renin produced by high albumin solutions was reversed by low-sodium perfusion. Furthermore, in experiments with high albumin perfusion, the inhibitory effect of low-sodium perfusions could in turn be reversed by restoring the osmolality of the medium with the impermeant solute, mannitol, even though extracellular sodium remained low.

The impression gained from these experiments is that the changes in the cell volume predicted by the varied maneuvers might be a critical common denominator influencing the secretion of renin. The fact that such marked changes are elicited in non-filtering kidneys suggests that alterations in the composition or rate of flow of distal tubular urine in relation to...
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the macula densa are not responsible for the phenomenon, though it is conceivable that the macula densa or other distal tubular structures might be affected indirectly by changes in the composition of peritubular plasma.

Previous experiments have examined the effects of varying concentrations of sodium and other solutes on renin release in intact kidneys with conflicting results. That changes in the volume of smooth muscle cells in the juxtaglomerular apparatus might alter renin release has been suggested by Young and Rostorfer (1973), who raised the osmolarity of renal arterial plasma acutely by infusing small volumes of hypertonic solutions of NaCl, dextrose, or urea into the renal artery of dogs. These infusions abruptly increased the release of renin into the renal vein. In other experiments, by other investigators, where renin release was previously stimulated, the infusion of hypertonic solutions of sodium chloride did not increase further the release of renin but rather decreased it. Such was the case in the experiments of Shade et al. (1972), where infusions of sodium chloride into the renal artery of ureteral-obstructed non-filtering dog kidneys resulted in a decrease in renin output; however, all of their dogs had been subjected to prior constriction of the inferior vena cava, as well as renal arterial clamping, so that the baseline output of renin was already elevated. Nash et al. (1968) also found that intrarenal infusions of hypertonic saline diminished the high level of renin secretion by intact filtering dog kidneys, when the output of renin had previously been stimulated. Isotonic changes in the concentration of sodium in the perfusate of isolated rat kidneys filtering normally (sodium substituted by choline) are said not to alter renin release (Fray, 1976). The effect of increased plasma oncotic pressure on the secretion of renin by intact, filtering dog kidneys was studied by Hall and Guyton (1976), who found that intrarenal infusions of hypertonic dextran or human serum albumin stimulated renin secretion while causing renal vasodilation. They ascribed their results to a tubular mechanism dependent on altered flow or composition of fluid in the distal tubule, an explanation that appears excluded in our experiments. The results of Young and Rostorfer (1973) and Hall and Guyton (1976) are consistent with those reported here and, in the context of those of Fray (1976), suggest that the increased release of renin after infusion of hypertonic or hyperoncotic solutions is the result of changes in the volume of the cells in the juxtaglomerular apparatus.

It is especially interesting that peritubular capillary oncotic pressure has been shown to modify tubuloglomerular feedback (Persson, 1979), since, like renin secretion, this phenomenon is thought to operate via the juxtaglomerular apparatus. Hyperoncotic perfusion of peritubular capillaries near the macula densa in Munich-Wistar rats increased the sensitivity of the single-nephron feedback pathway, suggesting that the activity of the juxtaglomerular apparatus was augmented. These results are also consistent with our own and are compatible with an interstitial pressure-volume receptor mechanism, as postulated by Persson et al. (1979).

In summary, renin release by the isolated perfused rat kidney is greatly enhanced by perfusion with a high concentration of albumin. The augmented release of renin is eliminated by ouabain and also by reducing the concentration of sodium in the perfusate. In the latter case, renin release is restored by increasing the osmotic concentration of the perfusate with the non-permeant solute, mannitol. These results suggest that renin release may be governed by some function of the volume of certain cells within the kidney, perhaps those of the juxtaglomerular apparatus.

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