THE EFFECT OF A NATRIURETIC DOSE OF ANGIOTENSIN ON RABBIT KIDNEY COMPOSITION

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SUMMARY

1. To obtain information about the mechanism of the natriuresis induced by angiotensin, its effect on the distribution of electrolytes, urea and water in rabbit kidneys was examined.

2. In five control experiments, after infusion of normal saline at a low rate for several hours, the animals were killed and their kidneys removed and analysed for Na, K, Cl, urea and water content at five levels from outer cortex to papilla. In five further animals, the infusion was changed to 1 µg kg⁻¹ min⁻¹ of angiotensin for 40 min. This induced a significant natriuresis.

3. Significant changes in tissue composition induced by angiotensin were reduction in the Na, Cl, and urea concentrations throughout the medulla and papilla, an increase in the water content in these regions and an increase in outer cortical Na and Cl concentrations. In the inner cortex the water content was increased and the urea content decreased.

4. Angiotensin produced similar changes in renal tissue in experiments in which the same protocol was used in eight adrenalectomized rabbits and in five rabbits with unilateral renal denervation. The changes seen were therefore ascribed directly to angiotensin and not to adrenal hormone release or sympathetic nerve stimulation.

5. The most likely interpretation of the results is that angiotensin inhibits Na reabsorption in the ascending limb of the loop of Henle. However, other explanations, including alterations in vasa recta blood flow, are possible.

INTRODUCTION

It has been known for some years that intravenous infusions of angiotensin can cause natriuresis in animals and man (Hughes-Jones et al., 1949; Langford, 1964; Healy et al., 1965; Louis & Doyle, 1965; Lameijer, Soghikian & de Graeff, 1966; Malvin & Vander, 1967). The mechanism whereby this is produced remains obscure. Early claims, based on clearance

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techniques (Hughes-Jones et al., 1949; Laragh et al., 1963; Langford, 1964; Malvin & Vander, 1967), that angiotensin inhibited tubular reabsorption of sodium have been doubted because of the limitations of these methods. Further, more direct methods of study of the effect of angiotensin on proximal and distal sodium reabsorption by micro-puncture technique (Horster et al., 1966) and in isolated proximal tubules from rabbit renal cortex (Burg & Orloff, 1968; Healy, Douglas & Arnold, 1969) have yielded negative results, although the possibility of a distal site of action gained some support from stop-flow studies (Vander, 1963).

The renal medulla is less accessible to these techniques. In this regard, Horster et al. (1966) unsuccessfully sought an inhibitory effect of angiotensin on sodium reabsorption in the loop of Henle by micro-puncture methods, but they did not establish whether a natriuretic response to the angiotensin occurred, and implied some difficulty in obtaining natriuresis with angiotensin under the conditions of micro-puncture. It has therefore not been established whether angiotensin inhibits sodium reabsorption in the ascending limb of the loop of Henle. Alternatively, a number of authors have suggested that the natriuresis induced by angiotensin may be due to alterations in intra-renal haemodynamics, particularly in the medulla, rather than a direct action on tubular reabsorption of sodium (Healy et al., 1965; Akinkugbe, Brown & Cranston, 1966; Early, 1966; Horster et al., 1966).

The present study was designed to seek further information about the mechanism of angiotensin-induced natriuresis by examining the effect of an intravenous infusion of a natriuretic dose of angiotensin on the distribution of electrolytes, urea and water in rabbit kidney slices. To exclude secondary effects due to release of catecholamine or aldosterone, or to central sympathetic nerve stimulation (Feldberg & Lewis, 1964; Staszewska-Barcsak & Vane, 1967; Biron et al., 1961; Scroop & Whelan, 1966), experiments were also done on adrenalectomized rabbits and in rabbits with the renal nerves divided.

METHODS

Conscious rabbits of 2–3 kg weight were used. Prior to the experiments the animals were maintained on a diet of standard animal pellets containing 26 mEq of sodium and 24·5 g of protein/100 g, and were kept in a normal state of hydration. Experiments were divided into three or four periods, all but the last of which were of 30 min duration; the last period was of 40 min. In control animals an infusion of normal saline at 0·0553 ml/min was given into an ear vein from a Harvard constant infusion pump. In angiotensin-treated animals the infusion was changed to 1 μg kg⁻¹ min⁻¹ of angiotensin in normal saline at 0·0553 ml/min for the last period. In nineteen of the twenty-three experiments urine was collected with an indwelling Foley catheter. The bladder was washed with 10 ml of distilled water and with air to ensure emptying. The rabbits remained quiet with minimal restraint throughout. At the end of the last period the rabbits were killed by exsanguination and the kidneys promptly removed and cut so as to obtain a longitudinal slice approximately ½ in. thick which incorporated the central papilla. Three segments of this were cut and each divided into outer cortex, inner cortex, outer medulla, inner medulla and papilla. Samples of tissue were immediately placed in pre-weighed acid-washed containers and were weighed without delay. The cutting and weighing took approximately 12 min for each kidney. To minimize evaporation, the second kidney was not cut until samples had been removed from the first kidney. Tissues were not frozen since Gardner & Vierling (1969) and Kobinger (1964) have found that this does not significantly affect the results.
Experiments were divided into three groups:

(i) In this group ten normal rabbits were used, of which five were controls and five received angiotensin.

(ii) Studies were repeated in eight adrenalectomized rabbits, four of which were controls and four received angiotensin.

(iii) Similar experiments were done in five animals with left renal denervation, all of which received angiotensin.

After the renal tissue was removed and weighed, one set of samples was dried overnight in a vacuum oven at 80° and the dry weight measured next day. Chloride was measured on these samples after the addition of 5 ml of 1.0 N HNO₃. Another set of samples was dissolved in concentrated HNO₃ and evaporated; sodium and potassium were measured on these after addition of a 6% lithium solution. The third set of samples was dried by gentle warming on a hot plate, the tissue ground in a mortar and suspended in 1 or 2 ml of water, depending on the size of the samples. After boiling for 15 min, urea and ammonia were estimated on these samples.

In nine experiments inulin was included in the infusion for the measurement of glomerular filtration rate (GFR), in which case blood samples for plasma inulin determination were taken from a vein in the ear opposite the infusion. In six experiments in which angiotensin was infused, arterial pressure was recorded throughout by means of a catheter in the femoral artery pushed up into the aorta and attached to a pressure transducer and a Hitachi QPD 54 recorder.

Chloride was measured with a Philips conductometric apparatus (PR9501), sodium and potassium by internal standard flame photometry, and urea and ammonia by Seligson's method (1965) employing urease and Nessler's reagent. Inulin was measured by the method of Walser, Davidson & Orloff (1955). Standard methods of statistical analysis were used (Snedecor, 1957).

Results were expressed as follows: tissue water, as l/kg dry weight; electrolytes as mEq/kg dry weight; and urea as m-mole/kg tissue water. Urea values were corrected for tissue ammonia levels.

Adrenalectomy was performed through bilateral flank incisions under pentobarbitone anaesthesia. The animals were subsequently maintained with a supplement of intramuscular DOCA in sufficient quantity to maintain body weight and normal serum electrolytes. Experiments were performed 1-2 weeks post-operatively.

Left renal denervation was carried out through a mid-line abdominal incision under pentobarbitone anaesthesia. The renal pedicle was exposed and the nerves and adventitia removed over approximately ½ in., leaving the artery and vein stripped. The animals were used for experiments 1-2 weeks later. At the end of the experiment the renal pedicles were removed and examined under a stereoscopic microscope with methylene blue stain for nervous tissue; it was clearly seen on the normal side, but was absent from the denervated side.

RESULTS

Preliminary comments

There was little difference between the results for the two kidneys in one animal. The coefficients of variation for measurements at a given level of tissue in opposite kidneys in
angiotensin-treated normal animals were small for sodium, chloride, potassium and water contents (5.3%, 8.0%, 8.8% and 7.6%, respectively). A coefficient of variation of 31.6% was obtained for urea content on the two sides but this was not large since the mean urea content was small (14.7 m-mole/l of tissue water) so that 31.6% of this was only 4.6 m-mole of urea/l of tissue water. Therefore the mean of the measurements for the two kidneys was used at each level in experiments in normal and adrenalectomized animals; in view of the good correlation between the two sides, in denervation experiments the operation was performed on the left side only and results from the two sides (denervated and innervated) were compared.

Urinary sodium excretion rates in control and angiotensin infusion periods are shown in Table 1. In experiments in normal animals, angiotensin caused natriuresis in the four experiments in which a catheter was used for urine collection, and a large diuresis was apparent in

<table>
<thead>
<tr>
<th>Table 1. Mean urinary sodium excretion rates in μEq/min during control and angiotensin infusions.</th>
<th>Where given, angiotensin was infused in Period 4. Range of results is given in parentheses below the mean. n = number of experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal series</td>
<td>Adrenalectomized series</td>
</tr>
<tr>
<td>Period</td>
<td>Control (n = 5)</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>(0.9-5.0)</td>
</tr>
<tr>
<td>2</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>(2.2-7.7)</td>
</tr>
<tr>
<td>3</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>(1.2-3.5)</td>
</tr>
<tr>
<td>4</td>
<td>2.1</td>
</tr>
</tbody>
</table>

* Single result.

the remaining animal. The mean increases in sodium excretion during the angiotensin infusion significantly exceeded the mean of all preceding control periods in the normal animals (P<0.05) and in the denervated series (P<0.01) but small numbers of urine collections precluded statistical significance in the adrenalectomized series. Unfortunately urine could not be collected in the fourth animal in the adrenalectomized series, but a large diuresis was apparent. It will be noted that one animal in the series with left renal denervation achieved only 3.9 μEq of sodium excretion per min during angiotensin infusion, which was nevertheless double its control excretion rate. The changes in tissue composition induced by angiotensin in that animal were of the same order as those seen in the remainder of that series and it was found that the degree of natriuresis was not significantly related to changes in tissue composition in any series. However, the mean natriureses with angiotensin in the three series were graded in relation to the extent of reduction in mean papillary sodium contents for the groups (see Tables 2, 3 and 4), i.e. mean papillary sodium was least in the series where the mean natriuresis was greatest.
Urinary urea concentrations in normal control animals varied between 13 and 46% of the papillary tissue urea concentrations. Urine urea concentrations measured in three of the angiotensin-treated animals were similar to papillary tissue urea concentrations (23.0 and 13.1; 14.7 and 13.8; and 20.0 and 19.9 m-mole urea/l of urine and tissue water, respectively). Urine urea concentration was not measured in other experiments.

During the course of this work, equipment became available which permitted blood pressure to be recorded in six experiments in which angiotensin was infused. These included two in adrenalectomized animals, two in unilaterally denervated animals and two extra experiments in which the same protocol was used in normal animals but in which the rabbits were not killed. Mean blood pressure (diastolic pressure plus one third of the pulse pressure) throughout control periods was stable and a fairly evenly maintained pressor response occurred with angiotensin. Average mean blood pressures at 15 min intervals in control periods were: 89, 88, 88, 89, 87 and 87 mmHg. In the angiotensin period they were 111, 117, 112 and 112 mmHg, at 10 min intervals. The average rise in mean blood pressure was therefore 25 mmHg, which was significant (P<0.001).

GFR was measured during the five experiments on rabbits with left renal denervation, in two experiments on adrenalectomized animals and in the two extra experiments in normal rabbits referred to in the last paragraph. In six of these nine experiments, angiotensin in the dose used reduced GFR; the mean results (±SD) for the nine experiments for three control periods were 10.2±3.7, 12.0±4.5 and 11.4±4.7 ml/min, respectively; the mean GFR fell in the angiotensin period to 9.2±3.2 ml/min, but this reduction was not significant.

**Effect of angiotensin on tissue composition**

Results of the studies in normal animals are summarized in Table 2. Angiotensin markedly reduced the medullary and papillary sodium, chloride and urea contents and increased the water content in these regions. In the cortex, angiotensin-treated animals had a higher sodium and chloride content in the outer zone. The water content of the inner cortex was increased, at which site urea content was reduced. The P values shown in this and succeeding series are based on the difference between the means of control and angiotensin-treated experiments.

In the adrenalectomized series the same pattern of results was seen, but the differences were less significant, partly because of the smaller series of experiments. The results are summarized in Table 3.

In the denervated series (Table 4) no difference could be found in the effect of angiotensin on the innervated and denervated kidneys. Comparisons of the results in this series with those for normal control animals showed that angiotensin caused the same pattern of response as was seen before: the sodium and urea values in the medulla and papilla were significantly reduced (sodium, \( P<0.05 \) to \( P<0.001 \); and urea, \( P<0.005 \) to \( P<0.001 \)). Since chloride concentrations followed sodium concentrations closely in all the preceding experiments, chloride was not measured in this series. Outer cortical sodium concentration was increased when compared with outer cortical sodium in control animals \( (P<0.05) \).

It will be noted that the results of tissue concentration of electrolytes and water are given per unit of dry weight. This shows absolute changes more accurately than the expression of results on a wet weight basis. If, for example, sodium had been expressed on a wet weight basis, the increase in measured tissue water in the medulla and papilla in angiotensin-treated animals would have resulted in an even lower figure. Some authors e.g. Saikia, (1965) prefer to
TABLE 2. Mean results of the effect of angiotensin on renal tissue composition in five experiments in normal animals compared with mean results of five control experiments in normal animals. SD shown below mean result

<table>
<thead>
<tr>
<th>Zone of kidney</th>
<th>Outer cortex</th>
<th>Inner cortex</th>
<th>Outer medulla</th>
<th>Inner medulla</th>
<th>Papilla</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na (mEq/kg dry weight)</td>
<td>246 ± 30</td>
<td>401 ± 40</td>
<td>743 ± 104</td>
<td>1279 ± 155</td>
<td>1603 ± 215</td>
</tr>
<tr>
<td>Cl (mEq/kg dry weight)</td>
<td>211 ± 25</td>
<td>343 ± 43</td>
<td>734 ± 108</td>
<td>1368 ± 163</td>
<td>1530 ± 158</td>
</tr>
<tr>
<td>Water (l/kg dry weight)</td>
<td>3-02 ± 0.10</td>
<td>3-27 ± 0.35</td>
<td>3-98 ± 0.04</td>
<td>4-79 ± 0.40</td>
<td>4-46 ± 0.54</td>
</tr>
<tr>
<td>Urea (m-mole/l of tissue water)</td>
<td>25-6 ± 16</td>
<td>41-2 ± 13</td>
<td>95-8 ± 32</td>
<td>237-2 ± 137</td>
<td>388-1 ± 181</td>
</tr>
<tr>
<td>K (mEq/kg dry weight)</td>
<td>339 ± 16</td>
<td>354 ± 13</td>
<td>404 ± 13</td>
<td>422 ± 27</td>
<td>389 ± 53</td>
</tr>
</tbody>
</table>

n.s., not significant; Contr., control; Angio., angiotensin treated animals.

TABLE 3. Mean results of the effect of angiotensin on renal tissue composition in four experiments in adrenalectomized animals compared with mean results of four control experiments in adrenalectomized animals. SD shown below mean result

<table>
<thead>
<tr>
<th>Zone of kidney</th>
<th>Outer cortex</th>
<th>Inner cortex</th>
<th>Outer medulla</th>
<th>Inner medulla</th>
<th>Papilla</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na (mEq/kg dry weight)</td>
<td>268 ± 57</td>
<td>466 ± 91</td>
<td>821 ± 138</td>
<td>1178 ± 186</td>
<td>1383 ± 190</td>
</tr>
<tr>
<td>Cl (mEq/kg dry weight)</td>
<td>288 ± 38</td>
<td>545 ± 80</td>
<td>888 ± 113</td>
<td>1243 ± 149</td>
<td>1452 ± 208</td>
</tr>
<tr>
<td>Water (l/kg dry weight)</td>
<td>3-28 ± 0.37</td>
<td>3-68 ± 0.39</td>
<td>4-22 ± 0.18</td>
<td>4-90 ± 0.57</td>
<td>4-81 ± 1.09</td>
</tr>
<tr>
<td>Urea (m-mole/l of tissue water)</td>
<td>20-2 ± 8</td>
<td>32-1 ± 14</td>
<td>53-8 ± 25</td>
<td>121-2 ± 53</td>
<td>232-1 ± 121</td>
</tr>
<tr>
<td>K (mEq/kg dry weight)</td>
<td>339 ± 20</td>
<td>367 ± 34</td>
<td>378 ± 29</td>
<td>404 ± 35</td>
<td>384 ± 35</td>
</tr>
</tbody>
</table>

n.s., not significant; Contr., control; Angio., angiotensin treated animals.
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express results as units per urea-free dry solids, since urea can contribute significantly to the total solids, particularly in the papilla. Such a correction here, however, would only have served to exaggerate the reduction in tissue sodium in medulla and papilla induced by angiotensin, as urea content was reduced also. The results are significant without such a correction.

Attention has recently been drawn by Gardner & Vierling (1969) to difficulties in interpretation of tissue water content in this preparation. When expressed on a dry weight basis,

<table>
<thead>
<tr>
<th>Zone of kidney</th>
<th>Outer cortex</th>
<th>Inner cortex</th>
<th>Outer medulla</th>
<th>Inner medulla</th>
<th>Papilla</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>Na (mEq/kg)</td>
<td>320</td>
<td>292</td>
<td>423</td>
<td>404</td>
<td>624</td>
</tr>
<tr>
<td>dry weight</td>
<td>±38</td>
<td>±35</td>
<td>±16</td>
<td>±38</td>
<td>±62</td>
</tr>
<tr>
<td>Water (l/kg)</td>
<td>3-65</td>
<td>3-45</td>
<td>4-44</td>
<td>4-16</td>
<td>5-66</td>
</tr>
<tr>
<td>dry weight</td>
<td>±0-22</td>
<td>±0-18</td>
<td>±0-30</td>
<td>±0-23</td>
<td>±0-32</td>
</tr>
<tr>
<td>Urea (m-mole/l of tissue water)</td>
<td>±6-2</td>
<td>±5-1</td>
<td>±1-7</td>
<td>±5-3</td>
<td>±5-5</td>
</tr>
<tr>
<td>K (mEq/kg)</td>
<td>329</td>
<td>329</td>
<td>376</td>
<td>381</td>
<td>419</td>
</tr>
<tr>
<td>dry weight</td>
<td>±9</td>
<td>±9</td>
<td>±19</td>
<td>±12</td>
<td>±22</td>
</tr>
</tbody>
</table>

Right, results from right (normally innervated) kidneys. Left, results from denervated kidneys.

tissue water may be overestimated by reduction of tissue solids, especially in the papilla. Thus the same amount of water when related to a lesser amount of dry weight may appear to have increased. Therefore, the increases induced by angiotensin in tissue water in medulla and papilla may be more apparent than real, due particularly to the sharp reduction in urea content, which contributes significantly to tissue solids in these regions. When the water content of the papilla in the experiments in normal animals was calculated on the basis of litres of water/kg urea-free dry solids, it rose in the control animals from 4-46 l/kg dry weight to 5-01 l/kg urea-free dry solids. On the other hand, correction for the small amount of urea in the papilla of angiotensin-treated animals made virtually no difference to the results, which then averaged 7-20 l/kg urea-free dry solids. It is possible that other unmeasured solids decreased in the papilla during angiotensin infusion, and correction for these materials may have elevated control papillary water content further towards the values of the angiotensin-treated animals.

DISCUSSION

The most prominent feature of the results was the reduction of the sodium and urea gradients in the medulla and papilla by angiotensin. This was evidently a direct effect of angiotensin on the kidney and was not due to secondary hormonal or nervous factors, since removal of the adrenal glands or the sympathetic nerves did not prevent the changes in tissue composition induced by angiotensin. A number of explanations for the results are possible.

Inhibition of sodium reabsorption in the ascending limb of the loop of Henle. Angiotensin may
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have interfered with the countercurrent system by inhibiting sodium reabsorption in the ascending limb of the loop of Henle. This would result in a decrease in the movement to the papilla of sodium and chloride and could account for the changes observed in tissue composition and for the natriuresis. This interpretation gains support from the fact that other substances which are believed to inhibit sodium reabsorption in the ascending limb have similar effects on kidney tissue composition. Thus, benzylhydroflumethiazide (BHF) causes reduction in the sodium and urea gradients in the medulla and papilla as well as an increase in the water content in these regions (Kobinger, 1965), changes which are analogous to the effects of angiotensin. Ethacrynic acid is also believed to inhibit sodium reabsorption in the ascending limb and it similarly reduces the sodium and urea content and increases the water content of the inner medulla and papilla (Goldberg et al., 1965).

The increase in medullary and papillary water content with agents that inhibit sodium reabsorption in the ascending limb is less readily explained than the other changes seen. However, this increase may to some extent be an artefact due to reduction in tissue solids. Nevertheless it is also possible that with inhibition of sodium reabsorption in the ascending limb, water would continue to diffuse into relatively hypertonic interstitium from the descending limb until the osmotic gradient disappeared, although limitation of solute movement from the loop would limit diffusion of water into the papilla. Medullary and papillary water content might also be increased by enhanced water reabsorption from the collecting ducts as a consequence of the presentation to the ducts of an increased volume of fluid during the diuresis. Whatever the explanation for the tendency for BHF and ethacrynic acid to increase tissue water, angiotensin clearly possesses the same property and may share with them a common site of action.

The increased sodium content induced by angiotensin in the outer cortex in the present study may be explained on the basis of inhibition of sodium reabsorption in the ascending limb of the loop. Although normally the distal tubules constitute about 10-20% of the volume of outer cortex (Roch-Ramel, Chomety & Peters, 1968), it is probable that if there is impaired sodium reabsorption in the medulla, then both the concentration of sodium in the distal tubules and the distal tubular volume may be increased, and augment the contribution of the distal tubule sodium to that in the outer cortex.

A similar explanation may be offered for the increase in cortical water, viz., distal tubule distention. The failure to detect elevation of sodium content in the inner cortex may be due to the higher normal sodium concentration found here than in the outer cortex.

The lack of effect of angiotensin on potassium content in renal tissue is no doubt due to most of the measured potassium being intracellular.

An inhibitory effect of angiotensin on sodium transport in the loop of Henle has previously been sought unsuccessfully by micro-puncture methods (Horster et al., 1966). However, there was no evidence that a natriuresis was induced during these experiments.

Increased vasa recta blood flow. The second possible explanation for the reduction of the medullary and papillary solute gradients and for the natriuresis is that angiotensin may increase blood flow in the vasa recta. In an attempt to explain the natriuresis consequent on increased vasa recta blood flow, Selkurt, Womack & Dailey (1965) have speculated that increased blood flow in those vessels would increase removal of sodium from the medulla and papilla; this would decrease water and solute reabsorption in the descending limb and increase flow in the ascending limb of the loop. The time for sodium reabsorption in the
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ascending limb would then be reduced and a natriuresis could result. Increased vasa recta blood flow has been proposed to explain the washout of medullary and papillary solute gradients by noradrenaline, supported in that case by $^{85}$krypton studies which also suggest that it increases medullary blood flow (Carrière, 1966). However, it is more difficult to account for the increase in tissue water content induced by angiotensin on this basis. It is possible that an increased volume of fluid in the loops of Henle plus a greater volume of blood in the vasa recta could have contributed to increased tissue water; but the amount of water in papillary tissue increased by 38%, which seems more than can be explained by distention of these structures. Further, the mode of death (exsanguination) might have reduced the volume of blood in the vasa recta.

There is also some evidence that medullary blood flow is not increased by angiotensin. Bonjour & Malvin (1969) have shown that angiotensin, in either natriuretic or antinatriuretic dosage, has no effect on the extraction ratio of para-amino hippurate in the rat kidney; since angiotensin reduced cortical blood flow, this would imply that the medullary blood flow was proportionately reduced. Autoradiographic studies of the effect of renin on rabbit kidneys have also suggested no change or a fall in vasa recta flow (Daniel, Prichard & Ward-McQuaid, 1954). On the other hand, Pomeranz, Birch & Barger (1968), on the basis of $^{85}$krypton washout curves have suggested that angiotensin increases the outer medullary blood flow, but this was only with an antinatriuretic dose. Studies of the effect of natriuretic doses of angiotensin on $^{85}$krypton washout in the medulla are not available.

Influence of increased blood pressure. The extent to which the pressor response to angiotensin was itself responsible for the changes in kidney tissue composition is difficult to assess, but a rise in blood pressure may produce changes in the kidney by increasing some portion of the renal blood flow or by increasing GFR. The angiotensin pressor response is associated with reduced cortical blood flow in the rabbit (Langford & Pickering, 1965) and as has already been discussed, it is improbable that it increases the medullary blood flow. Further, in our experiments and in those of others in rabbits (Langford & Pickering, 1965), GFR was not increased by this dose of angiotensin. It is difficult to accept that anything short of a measurable increase in GFR could wash out the medullary solute gradients to the extent that was found.

It seems likely that the effect of the pressor response to angiotensin might be important in inducing tissue changes if it increases medullary blood flow, although subtle, complex changes in intrarenal haemodynamics cannot be excluded. It is difficult to increase the blood pressure as a control to the angiotensin studies without introducing complicating factors; thus, other drugs, e.g. noradrenaline, may specifically influence medullary blood flow (Carrière, 1966) and mechanical increases in blood pressure, e.g. by expansion of blood volume, may result in suppression of proximal tubular sodium reabsorption (Rector et al., 1964).

Other explanations. Other less likely explanations of the results may be sought. Table 5 shows the effects of various agents on medullary and papillary tissue constituents (water diuresis, Levitin et al., 1961, and Roch-Ramel & Peters, 1967; mannitol, Malvin & Wilde, 1959, and Goldberg et al., 1964; 5% glucose and hydrochlorothiazide, Baer et al., 1962, and Goldberg et al., 1965; noradrenaline, Carrière, 1966; BHF, Kobinger, 1965; ethacrynic acid, 3% NaCl, and mercurial diuretics, Goldberg et al., 1965, and Heller, Škrhová & Vostál, 1965; chlorothiazide, frusemide and aminophylline, Heller et al., 1965; antidiuretic hormone, Gardner & Vierling, 1969). Those substances producing effects most like those of angiotensin
<table>
<thead>
<tr>
<th>Constituent</th>
<th>Angio.</th>
<th>WD</th>
<th>M</th>
<th>5% G</th>
<th>NA</th>
<th>BHF</th>
<th>EA</th>
<th>CT</th>
<th>F</th>
<th>3% NaCl</th>
<th>Hg</th>
<th>HCT</th>
<th>Am</th>
<th>ADH</th>
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<td>↓</td>
<td>OM nil P↓</td>
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<td>OM↑↓</td>
<td>IM and P nil</td>
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<td>P↑ ↑</td>
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<td>Water</td>
<td>↑</td>
<td>↑</td>
<td></td>
<td></td>
<td></td>
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</table>

All findings relate to absolute (dry weight) changes in Na content, except for F, where mEq Na/unit of wet weight was used. Dash indicates information not available. See text for sources of reference.

Abbreviations: Angio., angiotensin; WD, water diuresis; M, mannitol; 5% G, 5% glucose; NA, noradrenaline; BHF, benzylhydroflumethiazide; EA, ethacrynic acid; CT, chlorothiazide; F, frusemide; Hg, mercurial diuretics; HCT, hydrochlorothiazide; Am, aminophylline; ADH, antidiuretic hormone; OM, outer medulla; IM, inner medulla; P, papilla.
Angiotensin effect on kidney composition

are grouped to the left of the Table. Water diuresis, mannitol and 5% glucose all reduce the tissue sodium. This has been explained on the basis of a washout of sodium by the increased flow rate induced in the loop of Henle, but with the last two agents, increased medullary blood flow may contribute. If angiotensin similarly increased delivery of fluid to the loops, this mechanism might have been responsible for the observed effects. However, it would then be necessary to postulate that angiotensin either increased GFR or reduced proximal salt and water reabsorption.

The failure of angiotensin to increase GFR has already been discussed. It is unlikely that it has any inhibitory effect on proximal sodium reabsorption; angiotensin is without effect on sodium transport or intracellular electrolyte composition of isolated rabbit proximal renal tubules (Burg & Orloff, 1968; Healy et al., 1969). Further, Thurau et al. (1967) were unable to verify the results of Leyssac (1964), whose experiments on collapse time of rat proximal tubules following interruption of the circulation were said to demonstrate an inhibitory effect of angiotensin on proximal sodium reabsorption.

It has also been shown that ethacrynic acid, whilst having similar effects on medullary and papillary composition to those of angiotensin, did not reduce outer medullary sodium levels (Goldberg et al., 1965). This was ascribed to its capacity to inhibit proximal tubular sodium reabsorption, which resulted in a greater delivery of sodium to the outer medulla, apart from its effect in inhibiting sodium reabsorption in the ascending limb of the loop. Since angiotensin reduced the sodium level in the outer medulla, it would seem that it does not have such an effect on the proximal tubule. Table 5 also shows that the effects of angiotensin on tissue composition differ from those of the diuretic compounds listed on the right side of the table, viz. 3% NaCl, mercurial diuretics, hydrochlorothiazide and aminophylline. This suggests that the mechanism of natriuresis with these agents may be different from that with angiotensin. The first three of these diuretics are thought to decrease proximal sodium reabsorption, which increases the likelihood that angiotensin does not do so.

Further remarks. The findings of the present investigation may be compared with those of Ishii & Tobian (1969), who have studied the effects of hypertension induced by unilateral renal artery stenosis on the distribution of water and electrolytes in both the kidney exposed to the elevated blood pressure and the 'clipped' kidney. A number of changes similar to those reported here occurred, including reduction in medullary and papillary sodium in both kidneys and reduction in urea content in these regions in the 'unclipped' kidney. There was, however, no increase in the water content of the papilla. It is tempting to view their results as being due to the endogenous activation of angiotensin.

In conclusion, various possibilities have been considered to explain the changes in renal tissue composition induced by angiotensin. These include inhibition of sodium reabsorption in the ascending limb of the loop of Henle, increases in vasa recta blood flow, increases in GFR, decreases in proximal sodium reabsorption, and a complex haemodynamic effect of the pressor response. The first explanation appears the most likely.

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REFERENCES


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