RENAL AND HEPATIC INACTIVATION OF ANGIOTENSIN IN RATS: INFLUENCE OF SODIUM BALANCE AND RENAL ARTERY COMPRESSION

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SUMMARY

1. Rates of inactivation of \textit{asn}^1\textit{val}^5\textit{angiotensin II} by isolated perfused rat liver and kidney have been investigated in a variety of experimental conditions.

2. The liver inactivates angiotensin at a rate independent of sodium balance.

3. Sodium loading reduces and sodium depletion enhances the capacity of the kidney to remove angiotensin.

4. In the presence of a silver clip on one renal artery with contralateral kidney intact, the clipped organ destroys angiotensin at a normal rate, and the capacity of the contralateral intact kidney to inactivate the peptide is reduced.

5. The kidney with its renal artery constricted by a silver clip, when the contralateral kidney has been removed, has a reduced ability to destroy angiotensin.

Many attempts have been made to associate increases in blood pressure in experimental or naturally occurring hypertension with changes observed in the renin–angiotensin–aldosterone system. Repeated measurements have been made of kidney renin content, circulating renin and angiotensin and of aldosterone metabolism, but to date the evidence that any, or all, of these factors are critically involved remains conflicting (Page & McCubbin, 1968; Pickering, 1968; Lee, 1969). The possibility that changes in the activities of enzymes destroying angiotensin might be concerned in the development of high blood pressure has been considered previously in studies relating to the disappearance of the peptide in plasma or in tissue homogenates (Dexter, 1942; Bing, 1962; Hickler, Läuler & Thorne, 1963; Lagrue & Meyer, 1963; Biron, Landesman & Hunt, 1964; Birbari & Hickler, 1965; Itskovitz & Miller, 1966; Itskovitz, Dudrick & Dyrda, 1967). The results of these studies are of doubtful significance. The capacity of organs to destroy angiotensin is unlikely to be reflected accurately by work using tissue homogenates (Johnson & Ryan, 1968).

Vane and his colleagues have recently demonstrated the importance of organ vascular beds...
in the inactivation of angiotensin \textit{in vivo} and the relative unimportance of circulating angiotensinases (Vane, 1969). Vane's observations have been supported by Biron, Meyer & Pannisset (1968) and by Leary & Ledingham (1969). It is critical therefore to determine whether or not manoeuvres known to alter electrolyte balance or blood pressure can simultaneously alter the capacity of individual organs to inactivate angiotensin. This point has been investigated using the techniques of isolated organ perfusion in the rat.

\textbf{METHODS}

Female Sprague-Dawley rats in a weight range of 180–250 g were supplied by Carworth Europe, Alconbury, England.

\textit{Perfusion techniques}

Perfusion of the liver was performed as described by Hems, Ross, Berry & Krebs (1966) and of the kidney by the method of Nishiitsutsuji-Uwo, Ross & Krebs (1967) with certain modifications. Both the organs were perfused \textit{in situ} for periods of not less than 6 min and were isolated by ties placed around appropriate vessels. Rates of perfusion were measured in each experiment and varied between 3.5 and 9 ml min\(^{-1}\) g kidney\(^{-1}\) or 13 and 20 ml/g liver. No attempt was made to regulate perfusion rates outside these limits. Livers were perfused at a constant hydrostatic pressure of 23 cm water, and kidneys by pulsatile flow at pressures adjusted to 120–200/60–100 mmHg. Details of anaesthetic, perfusion medium, apparatus used and angiotensin bioassay have been previously described (Leary & Ledingham, 1969; Hems \textit{et al.}, 1966; Nishiitsutsuji-Uwo \textit{et al.}, 1967). By these techniques adequate oxygen to preserve biochemical function can be provided without added red cells (Ross, 1966). Red cells were therefore omitted from the perfusing medium in this series of experiments.

\textit{Addition of angiotensin}

In all experiments asparagine\(^1\)valine\(^5\) angiotensin II (asn\(^1\)val\(^5\)angiotensin II) was used. The peptide was added to the medium 3 min before an organ was included in the circuit, allowing time for equilibration to take place. The concentration of angiotensin initially presented to the organ was 133 ng/ml of perfusate. The time course of angiotensin inactivation by medium alone and by perfused kidney or liver was determined by bioassay of serial samples of perfusate taken at 2 min intervals. Destruction of angiotensin was expressed as ng/g wet weight of tissue per min, corrected to a standard flow rate per g wet weight of perfused tissue. Livers were weighed at the end of perfusion. In the case of the kidney the weight of the organ was taken to be that of the unperfused contralateral kidney or, in those experiments when one renal artery was constricted, the ischaemic kidney was weighed after the experiment and a correction made for the change in weight induced by perfusion.

Inactivation of angiotensin II by the isolated perfused liver and/or kidney was studied in groups of rats on the following regimes:

1. Standard small animal diet (Spillers Ltd, Banbury, England) with unrestricted access to tap water. The estimated daily intake of sodium was 1 mEq and of potassium 4 mEq per rat. (Liver perfusion in four and kidney perfusion in ten rats.)

2. Same standard diet with replacement of tap water by 1\% sodium chloride solution. Daily
intake of sodium was approximately 11 mEq and of potassium 5 mEq per rat. (Liver perfusion in five and kidney perfusion in eight rats.)

3. Standard diet and 2% sodium chloride solution to drink. Fluid intake was higher in this group and estimated daily sodium intake was 30 mEq, and potassium 4 mEq per rat. (Kidney perfusion in five rats.)

4. Standard diet, unlimited tap water, one renal artery constricted by the application of a silver clip, leaving the contralateral kidney intact. (Liver perfused in three, ischaemic kidney in five and intact kidney in five rats.)

5. Standard diet, tap water or 1% sodium chloride solution to drink, uninephrectomy. (Kidney perfusion in five rats drinking water and five taking 1% saline.)

6. Standard diet, tap water or 1% sodium chloride solution, with hypertension induced by renal artery clip and contralateral nephrectomy. (Liver perfusion in three rats; kidney perfused in five rats taking water and in five taking 1% sodium chloride solution to drink.)

7. Sodium depletion induced by a single peritoneal dialysis with 5% dextrose in water, followed by tap water to drink and a diet modified from that of Struyvenberg, de Graeff & Lameijer (1965) providing approximately 0.2 mEq sodium and 1.3 mEq potassium per rat per day. (Liver perfusion in two and kidney perfusion in seven rats.)

8. Same fluid and diet as Group 7, with supplements of sodium chloride bringing the sodium intake to 5 mEq per rat per day. (Liver perfusion in three and kidney perfusion in seven rats.)

The Struyvenberg diet of Groups 7 and 8 differed from the Spillers diet of Groups 1–6 not only in sodium and potassium content but also in the amount of other ions present, notably calcium.

In each of these groups of rats, constant experimental conditions had been maintained for not less than 2 weeks before perfusion was undertaken. Organs from the eight experimental groups were perfused in random order over a period of 6 months so that any influence of chance factors or improvement in technical performance could be minimized.

The blood pressures of the animals in all groups except 7 and 8 were measured. Conscious animals were warmed to 39° for 10–20 min and systolic pressure was recorded using a pneumatic tail cuff and a distal pressure transducer attached to an oscilloscope (Beilin, Garcia & Blackwell, 1969).

RESULTS

Blood pressure. All five animals in Group 6 were hypertensive (systolic pressure over 150 mmHg) within 2 weeks of surgery. Hypertension also developed in six of the thirteen animals in Group 4, but the other rats in this group were found to have systolic pressures between 110 and 140 mmHg. The blood pressures of animals in the other experimental groups examined were less than 120 mmHg systolic.

Effects of changes in sodium intake

Liver perfusion. After 6 min perfusion, the livers of animals taking 1 mEq sodium per day inactivated from 230.4 to 558.0 ng angiotensin II, mean 376.5 ± 78.4 (SEM) ng. These results were not significantly altered by giving rats 1% sodium chloride solution to drink (range
264.0 to 528.5 ng; mean 359.3±30.1 ng). These results are shown in Fig. 1. The livers of sodium depleted animals inactivated 313.4 to 329.8 ng with a mean of 321.8±8.0 ng which was not significantly different from their sodium replete controls (range 267.2 to 350.3, mean 297.6±26.4 ng).

![Graph showing liver perfusions: effect of salt loading on inactivation of angiotensin. Inactivation of \text{asn}^1\text{val}^5\text{angiotensin II} ng per g wet weight of tissue±SEM. Open circles represent animals of Group 2 given 1% sodium chloride solution (n=5) and closed circles rats of Group 1 taking a normal diet (n=4). There was no significant difference in angiotensin loss at 6 min between the two groups (P>0.45).]

**Kidney perfusion.** In contrast to the findings in the liver, Figs. 2 and 3 show considerable changes in the rates of inactivation of angiotensin by the kidneys of salt loaded or salt depleted animals compared with controls. The amount of peptide destroyed at 6 min was reduced by almost 50% in those animals which had drunk 1% saline and even more in those given 2% saline (Fig. 2). Renal inactivation of angiotensin by salt depleted animals (Group 7) was significantly more than that by animals from Group 8, whose diet was identical apart from sodium intake (Fig. 3).

It is apparent from Figs. 2 and 3 that kidneys of animals taking 0.2 mEq sodium in the Struyvenberg diet (Group 7) inactivated approximately the same amount of angiotensin as did those of rats on Spiller's diet taking 11 mEq sodium per day (Group 2). The potassium intake was four times greater in animals taking the Spiller's diet than those of Groups 7 and 8 and major differences in the content of other ions including calcium were also present.

**Kidney perfusion: effects of renal artery clip**

*Unilateral clip: contralateral kidney intact.* There was no significant difference between the rates of inactivation of angiotensin by kidneys with clipped arteries and kidneys of intact animals. The kidneys contralateral to those with the arterial clip, on the other hand, destroyed
considerably less peptide (Fig. 4). There was no detectable relationship between the presence or absence of hypertension and change in the capacity of the artery-clipped or contralateral kidneys to inactivate angiotensin.

*Unilateral clip with contralateral nephrectomy.* The effects of uninephrectomy alone were studied. The remaining hypertrophied kidney destroyed angiotensin at the same rate as did organs taken from animals with both kidneys intact. The effects of salt loading were also similar in these two groups (Fig. 5). However, if the solitary kidney had had an arterial clip applied the situation was different and the clipped organ destroyed much less angiotensin than control.

![Graph showing kidney perfusions: effect of salt loading on inactivation of angiotensin.](image1)

**Fig. 2.** Kidney perfusions: effect of salt loading on inactivation of angiotensin. Removal of asn¹val⁹ angiotensin II ng per g wet weight of tissue ± SEM. Closed circles represent control animals of Group 1 taking a normal diet (n=10); open circles animals of Group 2 given 1% sodium chloride solution (n=8); closed triangles animals of Group 3 given 2% sodium chloride solution (n=5). The 6 min loss of angiotensin was significantly decreased in both the saline groups compared with controls (P<0.0025).

![Graph showing kidney perfusions: effect of salt depletion on inactivation of angiotensin.](image2)

**Fig. 3.** Kidney perfusions: effect of salt depletion on inactivation of angiotensin. Removal of asn¹val⁹ angiotensin II ng per g wet weight of tissue ± SEM. Open circles represent animals of Group 8 on a normal salt intake (n=7); closed circles sodium depleted animals of Group 7 (n=7). The difference between the two groups was significant after 6 min perfusion (P<0.05).
FIG. 4. Kidney perfusions: effects of unilateral arterial clip with contralateral kidney intact on inactivation of angiotensin. Removal of asn^{1}val^{9} angiotensin II ng per g wet weight of tissue ± SEM. Closed circles represent control animals of Group 1 with both kidneys intact and taking a normal diet \((n=10)\), open circles experiments in which a kidney with artery clipped was perfused \((n=5)\) and closed triangles those experiments in which the contralateral unclipped kidney was perfused \((n=5)\). With a clip on one renal artery, the contralateral organs destroyed less angiotensin at 6 min than normal control kidneys \((P<0.0005)\) or than the organs with clipped arteries \((P<0.05)\). There was no difference between kidneys with arterial clip and normal control organs \((P>0.2)\).

FIG. 5. Kidney perfusions: effects of uninephrectomy and salt loading on inactivation of angiotensin. Removal of asn^{1}val^{9} angiotensin II ng per g wet weight of tissue ± SEM. Closed circles represent a control group on a normal diet with kidneys intact \((n=10)\), open circles uninephrectomized animals on the same diet \((n=5)\). Animals given 1% sodium chloride solution are represented by closed triangles (both kidneys intact, \(n=8\)) and open triangles (uninephrectomized animals, \(n=3\)). Loss of angiotensin at 6 min was significantly reduced by salt loading in both groups \((P<0.0025)\). Loss was not significantly different during perfusion of kidneys from the intact animals and from the uninephrectomy group, regardless of sodium intake.
kidneys or than kidneys similarly clipped with the contralateral kidney intact (Fig. 6). In the absence of the contralateral kidney the capacity of the clipped organ to destroy angiotensin could not be reduced further by increasing dietary salt intake.

![Figure 6](image)

**FIG. 6. Kidney perfusions: effects of unilateral nephrectomy and of arterial clip and salt loading on inactivation of angiotensin.** Removal of asp	extsuperscript{1}val	extsuperscript{2} angiotensin II ng per g wet weight of tissue ± SEM. Closed circles represent animals with unilateral nephrectomy alone (n=5), closed triangles animals with uninephrectomy, a Goldblatt clip and systolic BP 150 mmHg (n=5). Open circles represent uninephrectomized animals given 1% sodium chloride solution (n=3). Loss of angiotensin was reduced at 6 min in the presence of a Goldblatt clip (P<0.0025) or after salt loading (P<0.0025).

**Liver perfusion: effects of renal artery clip**

The isolated rat liver inactivated angiotensin II amide at rates unaltered by renal artery constriction (P>0.05). The perfused livers of rats with renal artery constriction and uninephrectomy removed 223.0–313.5 ng angiotensin in 6 min, mean 254.4 ng, SEM 29.6. The livers of rats with unilateral renal artery constriction and an intact contralateral kidney inactivated from 329.6–437.1 ng in 6 min (mean 380.9±31.1). In control animals the range was 230.4–558.0 ng (mean 376.57±8.4).

**DISCUSSION**

Perfusion of the isolated rat liver and kidney is a reproducible method of studying the rate of disappearance of angiotensin passing through these organs (Leary & Ledingham, 1969). The liver inactivates the peptide at rates which are not altered by sodium loading or depletion. In contrast the changes in capacity of the isolated kidney to inactivate angiotensin with altered sodium intake are great. Angiotensin destruction varied inversely with salt-intake in rats given the Spiller's diet (Groups 1–6) and in rats taking the Struyvenberg diet (Groups 7 and 8).

Kidneys from animals on the Struyvenberg diet containing 0.2 mEq sodium per day (Group C
7) inactivated less angiotensin in 6 min than those of animals on the Spiller’s diet of 1 mEq sodium per day (Group 1). In addition the kidneys of animals taking 5 mEq sodium in the Struyvenberg diet (Group 8) destroyed angiotensin at rates similar to animals on the Spiller’s diet taking 30 mEq sodium daily (Group 3). These inconsistencies deserve comment. Major differences in constituents of the two diets other than sodium may be important. Eggleston & Krebs (1969) have shown that 3-5 fold differences in the activities of pyruvate-kinase and α-glycerophosphate dehydrogenase can be induced in the livers of rats of various strains by alterations in diet. Enzymes destroying angiotensin may also be responsive to changes in intake of many dietary constituents other than sodium, and direct comparisons of angiotensinase activity between rats of Groups 1-6 (Spiller’s diet) and 7 and 8 (Struyvenberg diet) cannot properly be made.

In rats with one renal artery constricted and the other intact, considerably less peptide was removed by the intact kidney than by the ischaemic kidney, while ischaemic kidneys inactivated angiotensin at about the same rate as controls. In rats with one kidney removed and the remaining renal artery constricted by a silver clip the ischaemic kidney removed less peptide from perfusate than its controls and inactivation was not further reduced when these animals had been given saline to drink.

Bing (1962) showed that in the rat the capacity of kidney extracts to destroy asn¹val⁵angiotensin II was reduced when DOCA-salt or renovascular hypertension had been established. Blaquier et al. (1961) using similar methods and investigating the same peptide, were unable to demonstrate any difference in angiotensinase activity between normal rat kidneys and those taken from animals rendered hypertensive by arterial clip. Our findings do not support those of Blaquier’s group, but extend and largely confirm those of Bing.

The question arises as to whether the changes observed are of physiological or pathological significance or whether they are artefacts. Our studies were performed on organs taken from single strains of one species: high concentrations of angiotensin had to be used to achieve reliable bioassay results; the perfusing medium did not contain plasma or red cells, and the use of an anaesthetic could not be avoided. All these points must be taken into account in assessing the possible significance of the results. However, the difference induced in the behaviour of the kidney by the various experimental manoeuvres used were great, consistent and statistically highly significant. No such changes were observed in the liver experiments. The changes observed in renal inactivation of angiotensin are not limited to the synthetic peptide, asn¹val⁵angiotensin. Recent experiments indicate that the amount of naturally occurring angiotensin II free acids inactivated during kidney perfusion is significantly reduced in rats drinking 1% sodium chloride solution when compared with those given water (Leary, 1969).

The possible mechanisms by which the isolated kidney may inactivate angiotensin at widely differing rates depending on experimental circumstances is not clear. Disappearance of the peptide during perfusion could result from breakdown of the molecule by enzymes (Bakhle, Reynard & Vane, 1969; Leary & Ledingham, 1969) or by attachment to receptor sites in the perfused organs (Biron et al., 1968). The observation that inactivation of angiotensin by tissue vascular beds can be inhibited by edetic acid or dimercaprol (Leary & Ledingham, 1969; Bakhle et al., 1969) favours an enzymatic process, but does not distinguish with certainty between these two possibilities.

Alteration of the rate of removal of angiotensin by the kidney could reflect change in the
affinity of enzymes or binding sites for the peptide, or could reflect changes in the distribution of blood flow between areas of the kidney with different inherent capacities to inactivate angiotensin. Horster & Thurau (1968) have demonstrated differences in filtration rate between superficial and juxtamedullary nephrons of rat kidneys. In rats taking a standard diet containing approximately 1 mEq sodium/day superficial nephrons have a lower individual glomerular filtration rate than do juxtamedullary nephrons. This difference in individual nephron GFR and distribution of renal bloodflow is reversed if sodium intake is increased to 5 mEq/day. If renal angiotensinase were located mainly in juxtamedullary nephrons, animals with a normal sodium intake would inactivate angiotensin at a faster rate than those of salt-loaded animals, in which perfusion of juxtamedullary nephrons would be reduced. This redistribution of renal bloodflow could possibly be mediated by the changes in kidney renin content that occur in response to renal artery clipping and changes in sodium balance (Gross et al., 1964). This theory must remain unproven until the angiotensinase activity of superficial and juxtamedullary nephrons has been measured.

The changes in the capacity of the rat kidney to inactivate angiotensin are of potential importance in any assessment of the role of the renin–angiotensin–aldosterone system in states of altered electrolyte balance or blood pressure. Decreased removal of angiotensin in the kidney would induce increased local tissue concentrations of the peptide with a resultant rise in renal vascular resistance. The concentration of angiotensin in the renal venous blood and later in the arterial circulation would not necessarily be detectably altered. Reduced kidney blood flow with increased vascular resistance, particularly in the efferent arterioles, is a consistent finding in all states of high blood pressure (Goldring et al., 1941; Friedman, Selzer & Rosenblum, 1941; Bradley et al., 1950; Cargill & Hickam, 1949; Bolomey et al., 1949). The possibility exists therefore that tissue concentrations of angiotensin in the kidney could be altered critically in certain circumstances, with resultant renal vasoconstriction caused by angiotensin, but not reflected by alterations in circulating concentrations of renin, angiotensin or aldosterone.

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