Br. J. Pharmac. (1970), **39**, 357–372.

Renal tubular flow dynamics during angiotensin diuresis in the rat

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Summary

1. Tubular size and lissamine green transit times were measured in rat kidneys undergoing a diuretic response to angiotensin II (0·5 μg/kg per min), and compared with the changes observed during diuresis induced by osmotic diuretics, noradrenaline and chlorothiazide.

2. Angiotensin always caused a marked prolongation in proximal and distal tubular transit times; individual distal convolutions were coloured for prolonged periods, and lissamine green appeared in high concentration in distal tubules.

3. Marked changes were observed in superficial tubular calibre during a stable diuretic response to angiotensin. Where distal tubular diameter was normal for the rate of urine flow, proximal tubular volume was generally reduced. In a number of experiments, however, distal tubules were markedly dilated, and in these cases proximal tubular volume was also often increased. Angiotensin may therefore be capable of causing a degree of internal hydronephrosis in the rat kidney.

4. Prolongation of dye transit times, and the appearance of a concentrated lissamine green bolus in distal tubules, was suggestive of a decreased superficial nephron flow rate, indicating that the diuretic effect of angiotensin may take place only through deeper nephrons.

Introduction

Angiotensin II has been implicated in the control of sodium balance, since it is a potent stimulus to the release of aldosterone, and exerts its own anti-natriuretic effect on the kidney. Since peripheral blood renin (Brown, Davies, Lever & Robertson, 1964) and angiotensin II (Boyd, Landon & Peart, 1969) concentrations are elevated following dietary salt restriction, such a function would seem appropriate, although recent evidence indicates that animals actively immunized to angiotensin II respond normally to salt restriction and overload (Peart, 1969). Although angiotensin, infused intravenously in normal subjects in pressor and sub-pressor doses, reduces sodium and fluid excretion (De Bono, Lee, Mottram, Pickering, Brown, Keen, Peart & Sanderson, 1963), a diuretic and natriuretic response occurs in hypertensives (Nijensohn, 1957 ; Brown & Peart, 1962). The physiological significance of this reversal of the renal response is unknown ; the diuretic effect is not maintained on continued infusion of angiotensin (Brown, 1963) and so is unlikely to play a role in causing the hyponatraemia of severe hypertension (Brown, Davies, Lever & Robertson, 1966).

In experimental animals, a dose dependent duality of the renal response has been demonstrated, small amounts causing sodium retention and larger amounts natriuresis, in dogs (Lameijer, Soghikian & de Graeff, 1966), rabbits (Barraclough, 1965), and rats (Barraclough, Jones & Marsden, 1967). In the rat, however, a degree of expansion of the extracellular fluid volume must be achieved before the diuretic response can be elicited (Bonjour, Regoli, Roch-Ramel & Peters, 1968).

The mechanism of the natriuretic response in animals was the subject of the present investigation. The natriuresis is apparently due to an inhibition of tubular reabsorptive capacity, but angiotensin does not inhibit active sodium transport in isolated amphibian skin (McAfee & Locke, 1967) or isolated, perfused rabbit proximal tubules (Burg & Orloff, 1968). In addition, no inhibitory effect could be detected on renal carbonic anhydrase (Healey & Douglas, 1968) or Na-K activated adenosine triphosphatase activity (Bonting, Canady & Hawkins, 1964).

In view of the lack of antagonistic effect of angiotensin in isolated sodium transport systems, it is tempting to invoke a haemodynamic change in causing the response. The pressor effect is unlikely to be solely responsible for the diuresis, since no relation exists between the degree of natriuresis and the extent of the pressor response (Bonjour & Malvin, 1969), and since a natriuresis could be obtained in the rat with a subpressor dose (Peters, 1963).

The present study investigated the changes in superficial tubular calibre during angiotensin diuresis, since proximal tubular diameter has been related to reabsorptive rate (Gertz, Mangos, Braun & Pagel, 1965). Comparison of tubular volume and transit time data indicated a reduction in superficial tubular volume flow rate during angiotensin diuresis. Large increases in urine flow rate always caused marked tubular dilatation, but at low urine flow rates, a differential effect of angiotensin could clearly be demonstrated. The relationship between tubular volume and urine flow rate was therefore investigated over a range of diuresis induced by osmotic diuretics, noradrenaline and chlorothiazide.

Methods

Diuretic experiments have been concluded within 30 to 40 min, to exclude the effect of depletion of extracellular fluid. Experiments have been carried out (*a*) in non-diuretic rats given 3 ml of saline solution during surgical preparation, and (*b*) in rats undergoing mild diuresis, in which saline was infused continuously at 0·06 ml/min in addition to the 3 ml load. These animals were used in clearance determinations.

Preparation of animals

Male Wistar rats weighing 250 to 350 g were anaesthetized with Inactin (5-ethyl-5-(1-methylpropyl)-2-thiobarbituric acid) 100 mg/kg intraperitoneally. Following induction of anaesthesia, a further 5 mg Inactin was administered intravenously at completion of surgery, and anaesthetic did not generally have to be administered throughout the rest of the experiment. The animal was placed on a heated stage, the left kidney exposed *via* a longitudinal 2 cm flank incision and prepared for incident-light microscopic examination as described by others (for example Brenner, Bennett & Berliner, 1968). The renal surface was viewed through an intact capsule with a Leitz " Ultropak " microscope, and photomicrographs obtained on Kodak

High Speed Ektachrome film using a "Robot" camera and coupled electronic flash. The ureter was cannulated with a 5 to 6 cm length of fine polythene tubing (internal diameter 0·28 mm, external diameter 0·61 mm), of which the tip was passed to the level of the renal hilum.

Lissamine green was injected *via* a silicone rubber catheter in the right jugular vein. Drugs and infusions were given through polythene catheters in the femoral veins. Blood pressure was measured from a carotid or femoral artery using a Sanborn pressure transducer (type 267B) coupled to a Sanborn recorder and recorded in mmHg (1 mmHg≡1·33 mbar). Blood samples were obtained from a carotid artery. Urine was collected under liquid paraffin in small plastic tubes, and the urinary volume determined by weighing, without making correction for specific gravity. The distal end of the ureteral catheter was kept 2 to 3 cm below kidney level to assist flow through the catheter.

Experimental protocols

(*a*) *Non-infused rats.* Following an equilibration period of 1 h after completion of the preparation, control urine collections of 5 or 10 min were commenced for 30 min, and control transit times and photomicrographs obtained. Diuretic administration was then started, and transit times and photomicrographs repeated when urine flow had stabilized (successive urine collections not more than 10% different). Angiotensin and noradrenaline were administered at (0·5 μg/kg)/min, in saline solution infused at 0·06 ml/min, and in some experiments the effect of the saline infusion alone was investigated. Mannitol (15% solution in water) was administered at 0·22 ml/min for 20 min.

(*b*) *Saline-infused rats.* Animals were prepared as above, and plasma and urine blanks obtained. Inulin was given as a priming dose (100 mg/kg intravenously) followed by a sustaining infusion which delivered 2 mg/min in saline solution at 0·06 ml/min. Control urine collections were commenced 1 to 1·5 h later, when plasma inulin concentration had reached a stable level, and blood samples (0.3 ml) obtained at 10 min intervals. Each blood sample was immediately replaced with the same volume of donor rat blood (freshly drawn) from an animal which had been injected with heparin (500 u.) and inulin (100 mg/kg) and immediately bled out.

Diuretic infusions were angiotensin (0·5 μg/kg)/min, noradrenaline (0·5 μg/kg)/min and chlorothiazide 2 mg/kg intravenously followed by (2 mg/kg)/h which were each administered in saline solution at 0·06 ml/min. Dextrose (20% solution in distilled water) was given at 0·11 ml/min or 0·22 ml/min. Urine and plasma samples, transit times and photomicrographs were obtained as above during periods of stable urine flow.

Determination of transit time

Transit times were determined by a modification of the method of Gertz *et al.* (1965).

A constant volume bolus of about 0·03 ml of 10% lissamine green in normal saline was injected rapidly, and times recorded by marking on a kymograph. In

diuretic experiments with dextrose and mannitol, the bolus size was increased to 0·05 ml to permit good resolution of the tubular lumen.

Proximal transit time was recorded as the time between appearance of dye in peritubular capillaries, and colouration of the last of a group of late proximal convolutions. Distal transit time refers to the period between appearance of dye in the late proximal convolution, and colouration of the earliest and latest distal convolutions visible in the microscope field. This time gives an indication of flow velocity in the loop of Henle together with a variable length of distal tubule. One or two control transit times were recorded immediately preceding the test infusion, and the results were expressed as a ratio of experimental to control values.

Measurement of tubular diameters

Changes in proximal and distal tubular volume were recorded as described by Rector, Brunner & Seldin (1966), by tracing the tubular lumen from projected transparencies. In addition, the luminal diameter of distal tubules was measured directly with calipers, at least ten separate measurements being obtained for each value of diameter quoted, and the measurements converted into microns by reference to a micro-scale photographed and projected at the same magnification. Proximal tubular diameter was not measured directly, because the lumen is much less regular than that of the distal tubule, and the degree of change occurring was much smaller.

Chemical determinations

Sodium and potassium concentrations in urine were measured by flame photometry using an atomic absorption spectrophotometer (Unicam SP90), with reference to external standards.

Inulin was determined in plasma and urine by the method of Heyrovsky (1956) modified for a urine sample of 0·01 ml and plasma sample of 0·1 ml. Dextrose and lissamine green did not interfere with the determination.

Lissamine green clearance was measured in four rats before and during the infusion of angiotensin (0·5 μg/kg)/min. Lissamine green was given as a priming dose of 5 mg/kg followed by a sustaining infusion (2 mg/min). Urine and blood samples were collected as described for inulin clearance determination. Plasma was diluted in normal saline, and urine was diluted in water. Lissamine green was estimated colorimetrically by absorption at 625 nm.

Drugs used

All solutions were made up immediately before use:

Inactin (supplied by courtesy of Promonta Ltd., Constanz); lissamine green (G. T. Gurr Ltd.); angiotensin (Valine[5] angiotensin II amide; Hypertensin, Ciba); noradrenaline (Levophed; Bayer); chlorothiazide (Saluric; Merck, Sharp and Dohme). Saline solution contained the following milliequivalents of ions per litre: Na^+, 147; K^+, 4·2; Ca^{++}, 4·4; total Cl^-, 155·4.

Results

Experiments have been designed to investigate tubular flow characteristics over a range of urine flow rates. The level of diuresis produced by angiotensin and noradrenaline was increased by the continuous infusion of saline.

Urine flow rate and inulin clearance (C_{IN}) generally increased about 1·5 h after starting the sustaining infusion, but urine flow, C_{IN} and sodium excretion had reached stable levels when diuretic administration was started. Control experiments in which saline solution only was administered at a low rate (0·06 ml/min), showed no marked change in proximal (-6 to $+10\%$) or distal (-11 to $+33\%$) tubular volume, slight further increases in urine flow, and generally a slight reduction in proximal and distal transit times (Table 1).

Diuretic experiments with noradrenaline, mannitol, chlorothiazide and dextrose

In rats given 3 ml of Ringer solution but no sustaining infusion, urine flow averaged $0{\cdot}0049 \pm 0{\cdot}0030$ (S.D.) ml/min. The infusion of mannitol (5% solution at 0·22 ml/min) or noradrenaline (0·5 μg/kg per min at 0·06 ml/min) produced increases in urine flow ranging from 0·026 to 0·055 ml/min (mannitol), and 0·01 to 0·041 ml/min (noradrenaline). The dose of noradrenaline used increased mean blood pressure from 142 ± 20 mmHg to 155 ± 23 mmHg. Distal tubular volume increased slightly in both groups, but proximal tubular volume showed no change different from that occurring in the control group. Proximal and distal tubular lissamine green transit times were always decreased by noradrenaline (increased flow velocity) but were little changed by mannitol (Table 1). The concentration of lissamine green in distal tubules appeared to be reduced during the diuresis produced by these substances.

In animals infused continuously with saline, urine flow rates during diuresis were higher than in the previous group. Distal tubular volume increased during diuresis, and increases in proximal tubular volume were sometimes recorded at higher levels of urine flow (Table 2). When urine flow increased above about 0·05 to 0·06 ml/min, a definite distension of the whole kidney was noticed, as judged from the degree of racking of the microscope stage necessary to maintain the surface in focus. Average C_{IN} showed no significant change during noradrenaline and chlorothiazide diuresis, but a significant ($P = 0{\cdot}01$ to $0{\cdot}001$) decrease was observed during hypertonic dextrose diuresis, which also produced a greater increase in urine flow. Proximal and distal tubular transit times increased slightly in the dextrose infused groups, but showed little or no change in the noradrenaline and chlorothiazide treated groups (Table 2). In the latter animals, the lissamine green bolus size had to be increased to 0·05 ml to permit adequate observation of distal tubules.

Angiotensin diuresis

The onset of angiotensin infusion was marked by an increase in mean blood pressure (from a control value of 146 ± 14 (S.D.) mmHg to 185 ± 14 mmHg) which was accompanied by a simultaneous, rapid decrease in kidney size, marked blanching of the renal surface, and collapse of some or all of the superficial proximal convolutions. This phase was followed after 15 to 20 s by a reopening of proximal convolutions, and a lessening of the visible vasoconstriction in the kidney. In

TABLE 1. *Changes in tubular volume and transit times produced by noradrenaline and mannitol in rats with a low initial urine flow*

Diuretic	No. of expts.	V (ml/min)		Tubular vol. change (%)		Transit time (exp./control)		
		Control	Exp.	Prox.	Dist.	Prox.	Early dist.	Late dist.
Noradrenaline (0·5 μg/kg per min)	9	0·0064 (0·0040)	0·026 (0·014)	—1·4 (9·9)	+32·1 (32·8)	0·84 (0·10)	0·74 (0·14)	0·74 (0·19)
Mannitol (5% solution at 0·22 ml/min for 20 min)	5	0·0038 (0·0019)	0·038 (0·011)	+5·2 (7·3)	+83 (55)	1·05 (0·22)	0·99 (0·17)	0·96 (0·14)
Saline solution at 0·06 ml/min for 20 min	6	0·0065 (0·003)	0·0096 (0·0041)	+0·67 (5·9)	+5·3 (19)	0·88 (0·04)	0·80 (0·09)	0·70 (0·07)

Control urine flow (V) values represent mean of three collections of 5 or 10 min. Experimental values measured at time of photographing renal surface. Tubular volume determinations made by tracing lumen from projected transparencies. "Early" and "late" distal transit times obtained from appearance time of lissamine green in first and last visible distal convolutions (see text). Average values shown, together with S.D. in parenthesis.

TABLE 2. *Changes in tubular volume and transit times produced by various diuretics in rats undergoing mild saline diuresis (saline solution infused at 0·06 ml/min)*

Diuretic	No. of expts.	V (ml/min)		Tubular vol. change (%)		Transit time (exp./control)			C_{IN} (ml/min)	
		Control	Exp.	Prox.	Dist.	Prox.	Early dist.	Late dist.	Control	Exp.
Noradrenaline (0·5 μg/kg per min)	6	0·023 (0·010)	0·049 (0·021)	+12 (18)	+48 (50)	0·87 (0·16)	0·95 (0·20)	0·99 (0·20)	1·22 (0·24)	1·26 (0·28)
Dextrose (20% solution at 0·11 ml/min)	7	0·029 (0·007)	0·085 (0·023)	+10·3 (20)	+133 (101)	1·11 (0·14)	1·18 (0·31)	1·76 (0·41)	1·38 (0·21)	1·20 (0·21)
Chlorothiazide (2 mg/kg i.v.+ 2 mg/kg per hr)	6	0·022 (0·016)	0·058 (0·014)	+4·4 (7·1)	+56 (35)	1·07 (0·12)	1·02 (0·13)	1·05 (0·08)	1·24 (0·13)	1·16 (0·06)

Control urine flow (V) values represent mean of three collections of 5 or 10 min; experimental level measured at time of photographing renal surface. Inulin clearance (C_{IN}) values represent mean of determinations on four to six urine collections (5 min) in pre-infusion period (control) and throughout test infusion (exp.). Explanation of other values as for Table 1.

addition, some distal tubules were often observed to colour dark green, although no further lissamine green had been injected. Blood pressure declined after the initial response, to a stable level of 174 ± 11 mmHg. Urine flow was always reduced initially, but after a variable antidiuretic period of 3 to 10 min, increased to diuretic levels in about 90% of all experiments performed. Sodium excretion increased from a control value of $1{\cdot}3 \pm 0{\cdot}76$ to $9{\cdot}8 \pm 5{\cdot}3$ μequiv./min in the diuretic phase, which was significantly greater than that during noradrenaline diuresis ($0{\cdot}31 \pm 0{\cdot}26$ to $2{\cdot}55 \pm 2{\cdot}4$ μequiv./min). In the diuretic phase, C_{IN} was reduced in nine experiments, increased in one and unchanged in one.

One of the most profound and consistent features of angiotensin diuresis was a prolongation of proximal and distal tubular lissamine green transit times (Fig. 1). Transit times were difficult to determine accurately during angiotensin infusion, because flow often appeared to be virtually static in some distal tubules. The capillary appearance time of lissamine green was increased by angiotensin, from a control value of $2{\cdot}2 \pm 0{\cdot}5$ s to a mean value of $3{\cdot}1 \pm 0{\cdot}9$ s during diuresis. Proximal tubular lissamine green transit time was increased by angiotensin, even in the experiments in which inulin clearance for the whole kidney was not decreased (Table 3). Distal tubular transit times were greatly prolonged, some tubules colouring as much as 3 or 4 min after the intravenous dye injection. In addition, the dye bolus appeared in high concentration, and coloured individual distal tubules for an increased time (Fig. 2). Lissamine green clearance was measured in four rats, and was found to be reduced or unchanged during infusion of angiotensin (Table 4). The

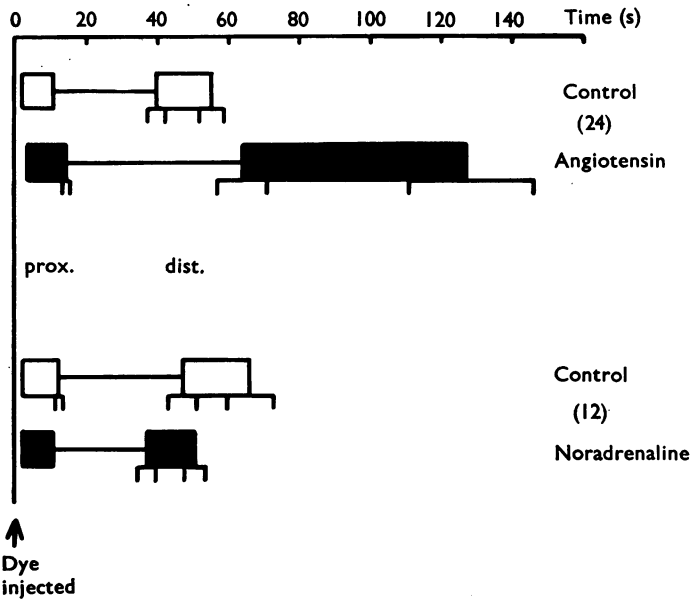

FIG. 1. Average changes in proximal and distal tubular lissamine green transit times during infusion of angiotensin and noradrenaline at (0·5 μg/kg)/min. Results shown from all experiments in which, during stable diuresis, urine flow ranged between 0·02 and 0·08 ml/min (angiotensin), and 0·01 and 0·08 ml/min (noradrenaline). Control values obtained in period immediately preceding test infusion. Standard error of mean shown where greater than 1 second. Figures in brackets indicate numbers of experiments.

reduction in renal clearance was accompanied by an elevation in plasma lissamine green concentration. Total plasma concentration was measured, and the results overestimated true lissamine green clearance, since the dye is known to be bound to plasma proteins (Grossman & Frey, 1969).

The diameter of superficial tubules varied markedly during angiotensin diuresis. Proximal and distal tubules dilated at the onset of diuresis, and the whole kidney

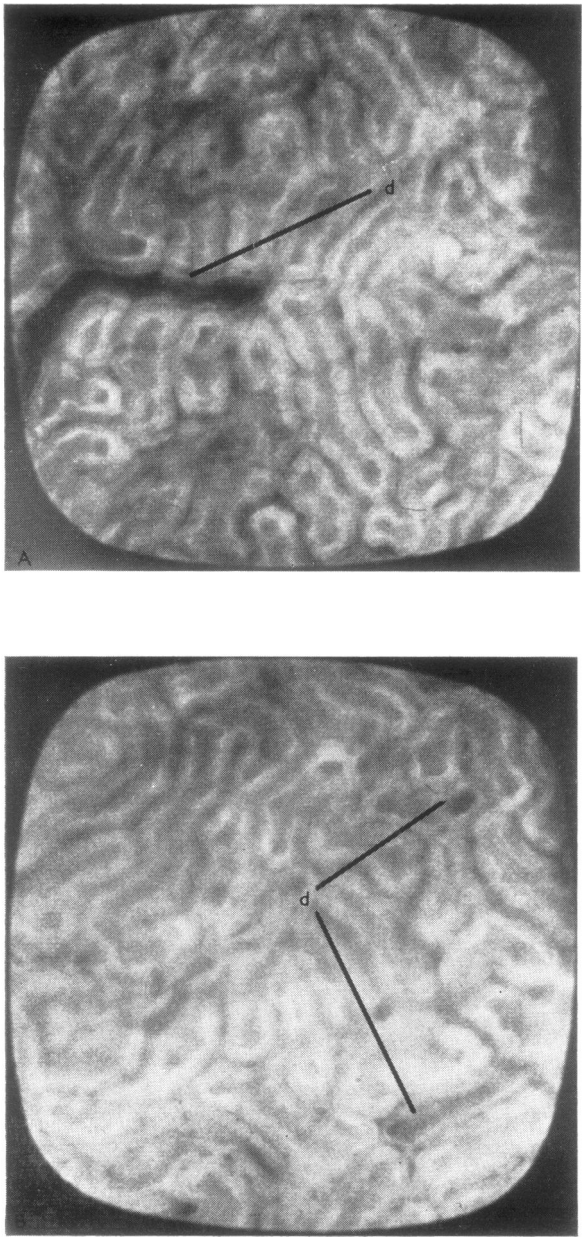

FIG. 2. Appearance of renal surface during angiotensin diuresis (A) at 0·047 ml/min, and noradrenaline diuresis (B) at 0·050 ml/min. Passage of lissamine green through distal tubules (d). (×*c.* 150.)

TABLE 3. *Changes in tubular volume and transit times during infusion of angiotensin II at* (0·5 *μg/kg*)/*min*

(a) *Rats not infused with saline*

Expt. No.	*V* (ml/min)		Tubular volume change (%)		Transit time (exp./control)		
	Control	Exp.	Prox.	Dist.	Prox.	Early dist.	Late dist.
5	0·0025	0·022	+39	+141	1·71	3·10	5·69
6	0·0064	0·047	+44	+156	1·00	1·30	2·10
7	0·0027	0·029	−15	+212	1·29	1·57	2·22
8	0·0017	0·026	+41	+309	1·48	1·75	2·48
62	0·0032	0·078	−19	+365	1·13	1·78	2·23
67	0·0057	0·047	− 8	0	1·44	1·44	2·28
68	0·0014	0·063	−13	+ 38	1·22	1·50	2·40
71	0·0037	0·030	− 8	+ 76	1·37	2·53	3·02
79	0·0047	0·047	− 1	+247	1·17	1·41	5·54
92	0·0024	0·050	+10	+110	1·33	2·00	3·58
Mean	0·0034	0·044	+ 7	+165	1·31	1·84	3·15
S.D.	0·0017	0·018	25	118	0·20	0·57	1·37

(b) *Saline solution infused at* 0·06 *ml/min*

Expt. No.	*V* (ml/min)		Tubular volume change (%)		Transit time (exp./control)			C_{IN} (ml/min)	
	Control	Exp.	Prox.	Dist.	Prox.	Early dist.	Late dist.	Control	Exp.
43	0·031	0·066	+25	+123	1·50	1·75	2·47	1·26	1·15
47	0·013	0·17	+16	+237	1·38	1·55	2·96	1·17	0·99
48	0·054	0·24	+63	+129	1·29	1·84	>2·50	1·10	1·26
50	0·018	0·11	+37	+137	1·39	0·82	1·59	1·26	1·17
53	0·013	0·052	+18	+295	1·43	1·22	2·64	1·51	1·26
77	0·044	0·096	− 4	+109	3·11	1·42	2·07	0·85	0·86
98	0·0053	0·060	+49	+452	2·02	3·40	3·92	1·23	1·06
99	0·025	0·033	−34	− 1	1·62	1·70	2·54	1·19	0·86
101	0·029	0·023	−37	− 30	1·00	1·10	1·63	1·44	1·21
102	0·0063	0·043	−32	+129	1·19	1·40	2·00	1·28	1·09
104	0·0090	0·059	0	+518	1·62	1·36	4·30	1·45	1·05
Mean	0·022	0·086	+ 9·2	+191	1·60	1·60	2·61	1·25	1·09
S.D.	0·016	0·066	34	172	0·57	0·67	0·91	0·18	0·14

Control urine flow values represent mean of three collections of 5 or 10 min; experimental level measured at time of photographing renal surface. Inulin clearance (C_{IN}) values represent mean of determinations on four to six urine collections (5 min) in pre-infusion period (control) and between 5th and 30th min of angiotensin infusion (exp.). Explanation of other values as for Table 1.

appeared distended. When urine flow had stabilized, however, distal tubules sometimes remained markedly distended, but in other cases did not appear more dilated than observed in diuretic experiments with other substances. Proximal tubular volume was often significantly reduced by angiotensin, but when a large increase in distal tubular volume had occurred, proximal tubules were often dilated, and the whole kidney appeared distended. Distal tubular transit time was prolonged to the greatest extent in those experiments in which distal tubules were obviously distended (see Table 3).

Measurement of changes in tubular volume did not give an absolute indication of the extent of tubular distension produced by angiotensin, since the change observed was dependent on the initial (control) level of urine flow. Distal tubular diameter was therefore measured directly from the projected transparencies and related to the urine flow determined simultaneously. During mannitol, chlorothiazide, noradrenaline and dextrose induced diuresis, as well as that occurring spontaneously, there was a linear relationship between urine flow rate and distal tubular diameter at urine flows of about 0·01 to 0·09 ml/min (Figs. 3 and 4). During

TABLE 4. *Renal clearance of lissamine green during infusion of angiotensin II* ((0·5 μg/kg)/min)

	V (ml/min)		Lissamine green clearance (ml/min)	
Expt.	Control	Expt.	Control	Expt.
A	0·0024	0·01	0·40	0·37
B	0·023	0·044	0·32	0·30
C	0·0016	0·0082	0·33	0·24
D	0·011	0·076	0·21	0·22

Figures represent mean of three 5 or 10 min urine collection periods, from mobilized left kidney.

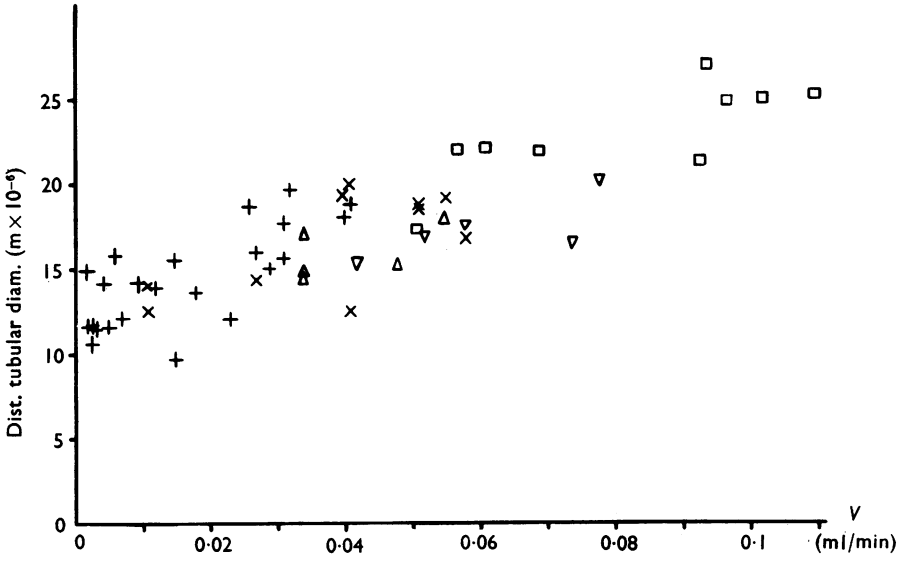

FIG. 3. Graph of distal tubular diameter against simultaneously measured urine flow rate (V). Results during spontaneous diuresis in pre-infusion periods (+), and during stable diuresis induced by infusion of noradrenaline (0·5 μg/kg)/min (×), chlorothiazide (2 mg/kg)/h (▽), mannitol (5% solution) at 0·22 ml/min for 20 min (△), and dextrose (20% solution) at 0·11 or 0·22 ml/min (□).

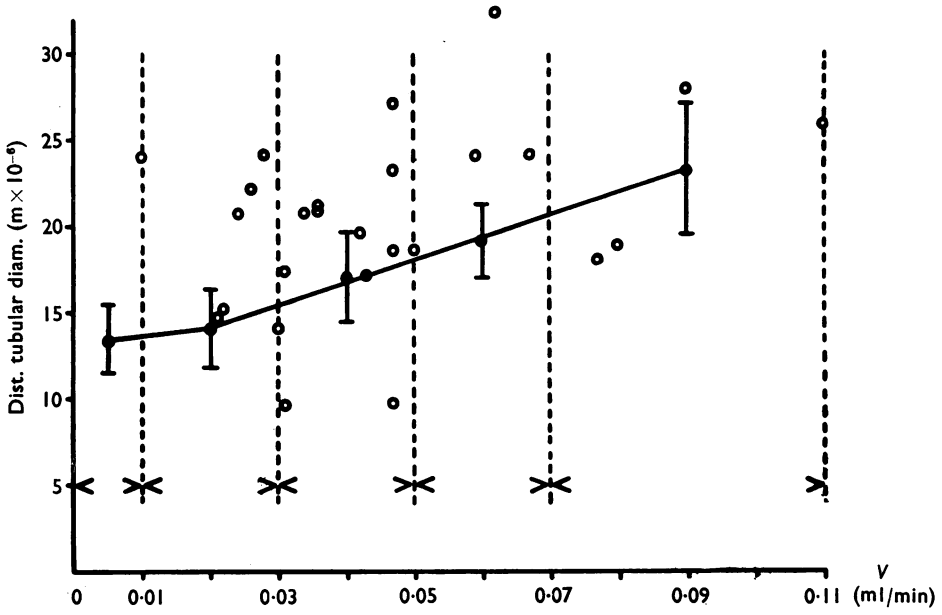

FIG. 4. Graph of distal tubular diameter against simultaneously measured urine flow rate (V) during stable diuresis induced by infusion of angiotensin at (5·0 μg/kg)/min (open circles). Closed circle symbols represent results from all other diuretic experiments presented in Fig. 3, averaged between urine flow rates indicated by dotted lines, together with S.D.

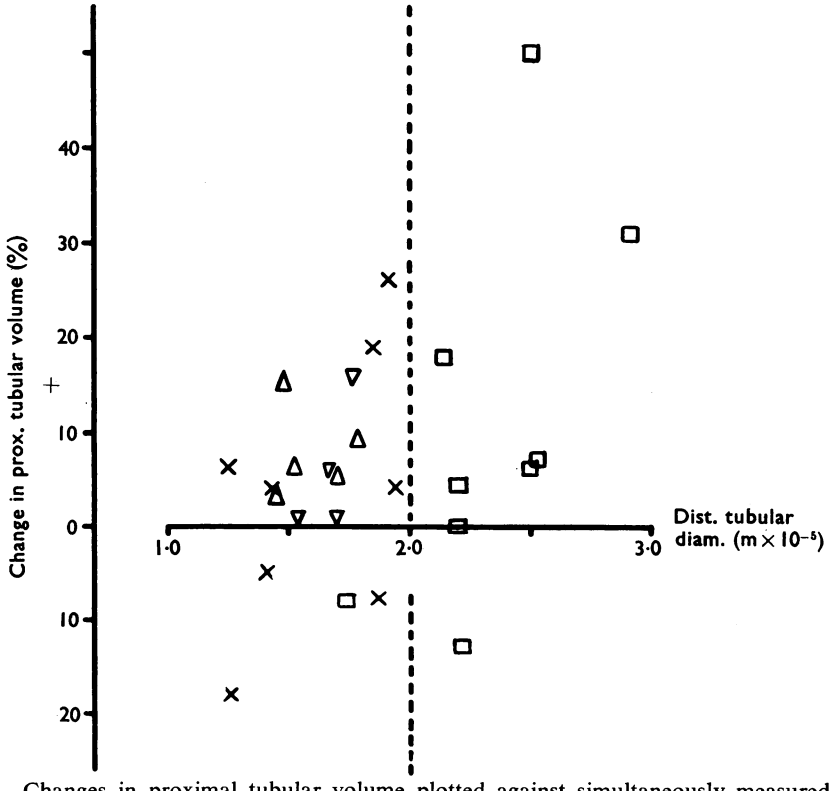

FIG. 5. Changes in proximal tubular volume plotted against simultaneously measured distal tubular diameter, at all levels of diuresis induced by infusion of noradrenaline, (0·5 μg/kg)/min (×); chlorothiazide, (2 mg/kg)/h (▽); mannitol, (5% solution) at 0·22 ml/min for 20 min (△); and dextrose (20% solution) at 0·11 or 0·22 ml/min (□). Arbitrary line of identity drawn through distal tubular diameter of 2×10^{-5}M).

angiotensin diuresis, however, the relationship between distal tubular diameter and urine flow was lost. Distal tubular diameter was sometimes greatly increased above the value expected for the level of urine flow, but in other experiments was equal to or even below the expected value (Fig. 4).

Changes in proximal tubular volume were related to the simultaneously measured distal tubular diameter. With the osmotic diuretics, noradrenaline and chlorothiazide, proximal tubular volume increased to the greatest extent at the greatest values of distal tubular diameter (Fig. 5). During angiotensin induced diuresis, proximal tubular volume was decreased at lower levels of distal tubular diameter, but an increase in distal tubular diameter above about 2×10^{-5} m was associated with an elevated proximal tubular volume (Fig. 6).

Discussion

This investigation revealed marked differences between the effects of angiotensin on renal function and tubular flow characteristics, and those of the other diuretic agents examined.

The initial blanching of the kidney and collapse of proximal tubules observed on commencing the angiotensin infusion is strongly indicative of afferent arteriolar

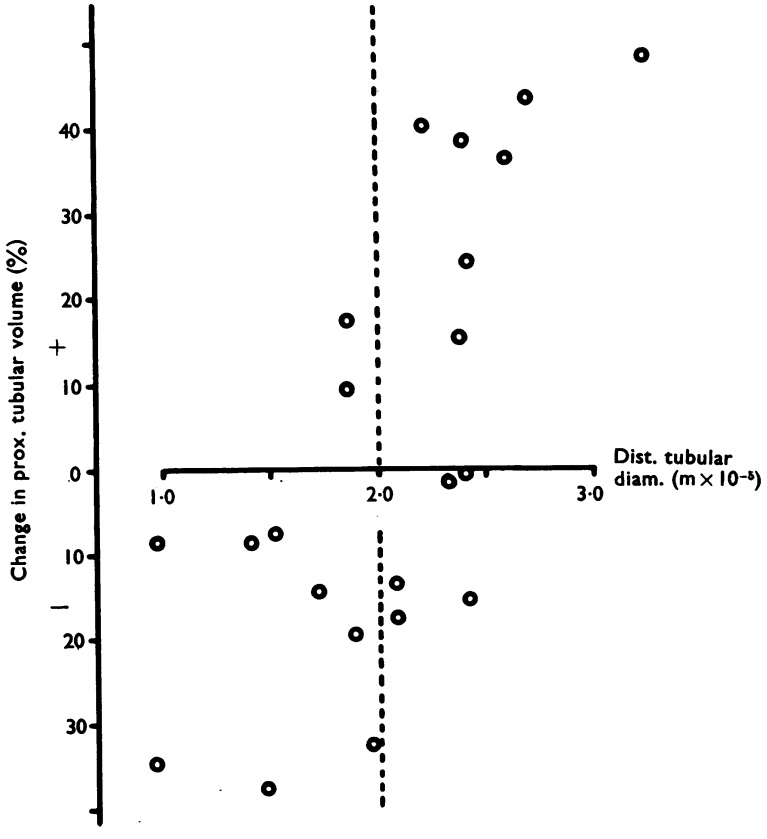

FIG. 6. Changes in proximal tubular volume plotted against simultaneously measured distal tubular diameter, at all levels of diuresis induced by infusion of angiotensin (0·5 μg/kg)/min.

constriction, and is similar to the effect reported by Leyssac (1965) following the intravenous injection of up to 25 ng of angiotensin. Leyssac also observed a reduction in proximal intratubular pressure following angiotensin injections. Such an afferent arteriolar constriction could provide a ready explanation for the reduction in glomerular filtration rate (GFR) of the whole kidney. The subsequent reopening of proximal tubules despite continued angiotensin infusion may indicate a tachyphylaxis to the effect of angiotensin of the smooth muscle of the afferent arteriole, but tubular perfusion may also have been modified by further changes in blood pressure, or other readjustments within the kidney.

A similar tachyphylaxis of the renal vasculature of the frog was observed by Wakim, Root & Essex (1941) following topical application of an angiotensin preparation. The first application produced blanching followed by engorgement of glomeruli, whereas repeated doses produced only engorgement.

The initial afferent arteriolar vasoconstriction to angiotensin, and reduction in GFR is presumably responsible for the preliminary antidiuretic period. Subsequently, a vascular adjustment occurs so that afferent and predominantly efferent arteriolar constriction takes place, as indicated by the rise in filtration fraction (Bonjour & Malvin, 1969); urine flow and sodium excretion may be increased or decreased while GFR remains reduced. The observation of a reduced GFR during a diuretic response to infusion of angiotensin at 0·5 μg/kg per min is in keeping with the results of Malvin & Vander (1967) in conscious rats.

Noradrenaline produced a smaller rise in blood pressure and a lower degree of diuresis and natriuresis than angiotensin, when infused at the same dose level. Both noradrenaline and adrenaline are known to be capable of producing diuretic responses in conscious rats (Dexter & Stoner, 1952 ; Green & Sim, 1961). When infused in equipressor amounts into cirrhotic subjects, angiotensin caused a considerably greater degree of natriuresis than noradrenaline (Laragh, Cannon, Bentzel, Sicinski & Meltzer, 1963). The mechanism of the diuretic response to noradrenaline is unknown, although the rise in blood pressure may be a greater contributory factor than in the case of angiotensin, since noradrenaline did not appear to produce such a marked degree of afferent arteriolar constriction.

Tubular radius has been used in this work as an indicator of transmural pressure changes. Distal tubular diameter showed a linear increase with urine flow, similar to the relationship between distal intra-tubular pressure and urine flow noted by Gottschalk & Mylle (1957). Distal tubular diameter presumably increases to a limiting value, which was not attained under the conditions of these experiments. The graph of tubular diameter against urine flow also shows that distal tubules remain open at zero urine flow, as has been observed by Steinhausen, Iravani, Schubert & Taugner (1963) following aortic occlusion above the renal arteries. The increase in proximal tubular volume observed at high urine flow rates is in keeping with the known elevation in proximal tubular hydrostatic pressure during osmotic diuresis (Gottschalk & Mylle, 1957).

During angiotensin induced diuresis, distal tubules were in some experiments greatly dilated, but in others, the diameter was normal for the rate of urine flow pertaining. Intratubular pressure was not measured directly in these expericents, and so the changes in diameter cannot be ascribed directly to changes in intratubular pressure. However, in those experiments in which tubules were dilated, the distension of the whole kidney was strongly indicative of the development of a degree

of internal hydronephrosis. The increase in proximal tubular diameter in angiotensin experiments was unlikely to be caused by the elevation in systemic pressure, since proximal tubular and peritubular capillary pressures remain constant over a wide range of spontaneous variation in blood pressure (Gottschalk & Mylle, 1956 ; Thurau & Wober, 1962). Koch, Aynedjian & Bank (1968) observed an increase in proximal intra-tubular pressure, and tubular dilatation, when systemic pressure was elevated by bilateral carotid occlusion and vagotomy in the rat ; in their experiments, however, carotid occlusion resulted in a large increase in urine flow which would be expected to cause a rise in intratubular pressure.

Distension of distal tubules produced by angiotensin indicated that, in some cases, resistance to flow in the collecting ducts was elevated. Such an elevation in collecting duct resistance could be due to increased medullary blood flow, resulting in an increased intravascular volume. However, the results of blood flow distribution studies indicated a reduction in medullary blood flow during angiotensin infusion (Finberg, 1969). Alternatively, collecting duct resistance could be elevated by constriction of the pelvis muscle (Finberg & Peart, 1970).

The reduced proximal tubular calibre produced in other angiotensin experiments was presumably a result of the reduced GFR. An acute reduction of GFR by aortic occlusion has been shown by others (Wahl, Liebau, Fischbach & Schnermann, 1968 ; Baines, Gottschalk & Leyssac, 1968) to result in a reduced superficial proximal tubular diameter. Conversely, the observation that proximal tubular diameter could be elevated even at relatively low rates of angiotensin diuresis makes untenable the hypothesis that the diuresis results from tubular compression, as was suggested to occur in vasodilated dog kidneys (Earley & Friedler, 1966).

The profound prolongation of proximal and distal tubular transit times caused by angiotensin resembles the effect of renal artery constriction in non-diuretic rats (Gertz *et al.*, 1965 ; Brenner *et al.*, 1968). In several experiments in which angiotensin caused an increased urine flow rate, distal transit time increased more than the simultaneously measured distal tubular volume (Table 3), indicating that distal tubular flow rate was lower than in the pre-infusion period. Comparison of transit time and cross-sectional area changes in this way can only yield an approximate estimate of flow rate changes, since the distal transit time refers to loop of Henle passage as well as the distal tubule, and loop distension cannot be estimated. However, the profound increases in transit time in experiments where tubular volume was not greatly increased (for example, Nos. 67, 68 and 71 in Table 3) lend greater significance to the presumed reduction in distal flow rate. The increased dye density in distal tubules, and very low flow velocity, also provide evidence for a reduced superficial nephron flow rate during the diuretic phase, so that the diuresis must take place through nephrons situated in deeper parts of the cortex. Lissamine green clearance by the whole kidney decreased during angiotensin infusion, showing that the dye was not concentrated by secretion, but that the increased dye density probably represented increased reabsorption of tubular fluid. It should be borne in mind, however, that the density of lissamine green colour is dependent on the pH of the medium (Grossman & Frey, 1969).

Individual nephron GFR has recently been shown to vary markedly throughout the kidney according to different conditions of salt and water balance. Horster & Thurau (1968) suggested that an increased filtration through deeper, juxtamedullary glomeruli would lead to salt retention, since filtrate would pass through longer

loops of Henle. Angiotensin may inhibit distal tubular reabsorption, although such an effect has not yet been demonstrated adequately by micropuncture techniques. Horster, Nagel, Schnermann & Thurau (1966) were unable to detect any effect of angiotensin on proximal tubular split-drop reabsorption time, or on sodium and water flux in microperfused loops of Henle ; however, the peptide was administered in conditions which were stated not to affect urine flow or sodium excretion. A distal site of action was inferred by Lowitz, Stumpe & Ochwadt (1969), following peritubular capillary perfusion of angiotensin in high concentration. These workers, however, mention that proximal tubules were occasionally seen to collapse following the micro-infusion, indicating that some of the dose was reaching glomerular arterioles, and that the effects observed could have been due to general haemodynamic changes rather than a specific effect on reabsorptive capacity. The results of the present study show that micropuncture analysis of superficial tubular fluid may not reveal the site of diuretic action of angiotensin in the rat.

If distal tubular reabsorption is inhibited by angiotensin, diuresis may result from an increased filtration through juxtamedullary glomeruli, but a markedly reduced filtration through superficial glomeruli leading to a net reduction for the whole kidney. The prolongation of transit time may therefore only refer to superficial tubules, and may be a cause of increased reabsorption in these nephrons. Alternatively, recent work in this laboratory suggests that angiotensin may exert a variable effect on sodium reabsorption in nephrons in different parts of the kidney, by an alteration in regional blood flow distribution (Finberg, 1969). The results of this work will be presented fully elsewhere.

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(*Received March* 4, 1970)