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THE NATURE OF THE ACTION OF RENIN AND HYPERTENSIN ON RENAL FUNCTION IN THE RABBIT

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In a previous paper (Pickering & Prinzmetal, 1940) it was shown that renin has a remarkable effect on urine flow in the rabbit. After intravenous injection the flow of urine was reduced during the first 10 min., and then rose to very high levels, as much as 50 ml. being produced in a half-hour period. The glomerular filtration rate, as measured by creatinine clearance, was found to fall during the antidiuretic phase, and to return to normal during the period of diuresis. The urine formed during the diuretic phase was remarkable in that its content of sodium and chloride tended to approximate closely to, and in the case of chloride slightly to exceed, that of plasma; and this was true whether the initial values of chloride were high or low. On the other hand, urea excretion was not dissimilar to that observed in a water diuresis of comparable degree. Since that work was done it has been shown that renin has the properties of an enzyme, acting on a constituent of the plasma globulins (hypertensinogen, or renin substrate) to form a substance of small molecular size, hypertensin (Braun-Menendez, Fasciolo, Leloir & Munoz, 1940) or angiotonin (Page & Helmer, 1940). As far as vascular effects are concerned, the action of renin seems to be entirely due to the formation of hypertensin; the actions of the two are qualitatively similar, and in the absence of hypertensinogen renin has no action on the blood pressure or the vessels of the perfused rabbit's ear (Page & Helmer, 1940). It therefore seemed of interest to inquire whether hypertensin would produce effects on renal function in the rabbit similar to those produced by renin. The opportunity has also been taken to study more closely the mode of action of these substances.

METHODS

The methods were essentially similar to those used in a previous publication (Pickering & Prinzmetal, 1940). Urine was obtained by catheter at intervals usually of 30 min. from unanaesthetized male rabbits (2 to 2·5 kg.) which were

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lightly held on their backs on the lap, the bladder being washed out with 5 ml. water in each instance. The room in which the experiments were done was kept warm (25–26°) to ensure dilatation of the ear vessels. All injections were given into, and all blood samples taken from, the left ear which had had its sensory nerves cut by previous aseptic operation; for these purposes the rabbit was taken from its cage and placed on a warm pad on which it was lightly secured with a cloth. Between catheterizations, the animals were placed in small wire cages in which they could turn round with difficulty. In these experiments urine was not voided naturally between the catheterizations. The rabbits were fed on a mixed diet, starved for 12 hr. previously but allowed access to water. Unless otherwise stated, all the experiments in this paper were during the subsidence of water diuresis induced by giving 100 ml. water by stomach tube some 3 hr. before urine collections were begun.

Renin and hypertensin. Renin was prepared from rabbit's kidney by a method previously described (Pickering & Prinzmetal, 1940). Alcohol-dried kidney powder was extracted with saline, clarified by acidifying to pH 5 and centrifuging, and the supernatant fluid half saturated with $(NH_4)_2SO_4$. The precipitate was dialysed till free of sulphate and the solution dried by the lyophil process. The yellow powder, completely soluble in saline, was used as required.

Hypertensin was prepared from ox hypertensinogen and rabbit renin by a method based on that of Braun-Menendez *et al.* (1940). Ox serum was half saturated with $(NH_4)_2SO_4$. The precipitate, separated by centrifuging, was dialysed till free of sulphate and filtered. Hypertensinase was destroyed by acidifying to pH 4·2 for 20 min. at 25° C. After neutralizing, the hypertensinogen solution was brought to 37° C., and to it was added an amount of renin previously found in pilot experiments to give a maximum yield of hypertensin. An amount of renin corresponding to 10 g. fresh rabbit's kidney was used for the hypertensinogen derived from 300 ml. ox serum. After incubating for 20 min., the mixture was poured into 4 vol. of a warm mixture of 9 vol. ethanol and 1 vol. ether, and filtered. The filtrate was distilled *in vacuo* at 50° C., and finally evaporated to dryness on a water-bath in air. The brown residue was taken up in saline to give a turbid solution which was clarified by shaking with ether. After separating, the watery layer was freed from ether on the water-bath. In some preparations a further precipitation with 4 vol. of absolute alcohol was carried out, and the filtrate evaporated to dryness and taken up again in normal saline.

Controls for hypertensin were prepared in the following way at the same time as two of these preparations. After destroying hypertensinase, a portion of the hypertensinogen was put through the process just described save that no renin solution was added before incubating at 37°. The final solution was made up to a volume which bore the same relation to the volume of the original

hypertensinogen solution as did the final volume of the hypertensin preparation. Thus the control (hypertensin control) should have contained all the substances in the same proportions as did the hypertensin solution, except hypertensin itself.

Inulin and diodone clearances. 1 g. of purified inulin in 10 ml. saline, and 5 ml. of a 35% solution of diodone were injected subcutaneously in the back and flanks just before, or just after, the administration of water. Clearances were done 4–6 hr. later, although in some cases after 6 hr. the plasma diodone had fallen too low for reliable results. Heparinized blood samples were obtained before injection of inulin and diodone, and in the middle of each period of urine collection. Both blood and urine samples were analysed by the methods of Hubbard & Loomis (1942) and Alpert (1941) with a slight modification in the case of diodone (Sanderson, 1948). The methods of estimating sodium and chloride were those of Morton (1945) and Sendroy (1937) respectively.

Glucose Tm. In male rabbits anaesthetized with intravenous nembutal (sodium pentobarbitone) a priming dose of 20 mg./kg. body weight creatinine and 0·9 g./kg. glucose in 6 ml. water was given intravenously, followed by a continuous intravenous infusion. In the first four experiments of Table 4, a solution containing 2·2 g. glucose and 70 mg. creatinine per kg. body weight in 100 ml. water was infused at a rate of 80 ml./hr. In the last two experiments of Table 4 a solution of creatinine and glucose four times as strong was given at 20 ml./hr. A catheter introduced into the bladder per urethram was tied in; urine was obtained at 10 min. intervals, the bladder being washed out with 5 ml. water and 5 ml. air. Heparinized blood samples were obtained from the dilated ear veins in the middle of each clearance period. Clearances were not begun until at least 25 min. after the infusion had begun.

Berlin blue injection of kidneys. The rabbit was anaesthetized with nembutal and tied on a table. The belly was opened ventrally, the abdominal aorta tied at its bifurcation and the left renal pedicle carefully exposed without touching the vessels. 10 ml. 2% soluble Berlin blue were injected in 10 sec. through a wide needle into the aorta just headwards of the ligature. As the last 2 c.c. were being injected the left renal pedicle was abruptly clamped in artery forceps, the ureter was next clamped and the kidney excised with the clamps attached. After fixing in formalin, transverse sections cut freehand at about 0·5 mm. were cleared in methyl salicylate and observed under the binocular microscope. Stained sections prepared from paraffin blocks were also examined. In four rabbits renin was injected intravenously after anaesthetization; 10 min. later the belly was opened and the aorta exposed ready for injection. In all these experiments the ureters were widely dilated with urine and the bladder filled quickly. It may be assumed, therefore, that at the time of injection renin was exerting its customary diuretic effect.

RESULTS

The effect of hypertensin. In a given rabbit the rise of arterial pressure produced by intravenous injection of hypertensin is, as with renin, roughly related to the logarithm of the dose. With both renin and hypertensin, however, the quantitative relationship differs from rabbit to rabbit. We have not attempted in these experiments to define the dosage in terms of arbitrary units, but we have adjusted the dose of the four preparations of hypertensin used so that they gave the same order of response, namely rises of arterial pressure varying from 25 to 50 mm. Hg in the different rabbits. With such doses the rise reaches its peak in 35–60 sec. and lasts 2–5 min. (see Fig. 1). As with renin, and in contrast to adrenaline and tyramine, responses of this order are unaccompanied by macroscopic change in the ear vessels of the warm rabbit.

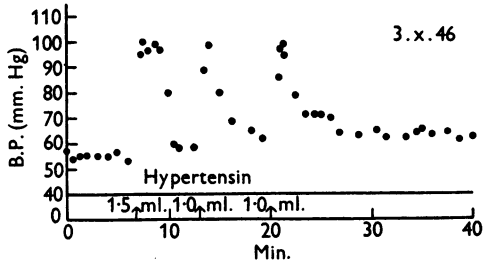

Fig. 1. Effect of the doses of hypertensin here employed on the unanaesthetized rabbit's blood-pressure. The rabbit and hypertensin preparation were the same as in Fig. 7; the time 24 hr later.

To determine whether hypertensin has a diuretic effect, it was injected towards the end of the falling limb of the curve of water diuresis. Urine collections were begun about 3 hr. after the hydrating dose of water, and continued for at least three half-hour periods, and until it was clear that the rate of urine formation was decreasing and until the volume per half-hour period was less than 10 ml. In these circumstances it is the rule for diminishing rate of water excretion to be accompanied by rising urinary chloride percentage, the rate of excretion of chloride remaining nearly constant (Figs. 6–8). Hypertensin was then injected intravenously in three doses at 3 min. intervals and the half-hourly collections continued. In all sixteen such experiments on ten rabbits and using three separate preparations of hypertensin, the volume of urine increased during the half-hour after the injection of hypertensin, the increase continuing into the next half-hour in five rabbits. The urine volume in the third half-hour after injection was less than the pre-injection level in all. These experiments are summarized in Table 1. As with renin a very conspicuous rise in chloride excretion accompanied the diuresis, this being due chiefly to increase in the urinary chloride concentration in these experiments in which this was initially

low, for in these animals no sodium chloride above that contained in the diet had been given by mouth (Fig. 2).

TABLE 1. Urine flow and chloride excretion in five successive half-hour periods of which two (1, 2) preceded and three (3, 4, 5) followed intravenous injection of hypertensin and hypertensin control

	Before hypertensin		After hypertensin		
	1	2	3	4	5
(1) Rabbits not previously given salt, ten experiments, nine rabbits					
Urine flow (ml./min.)	0·18	0·135	0·37	0·10	0·035
Cl′ excretion (μequiv./min.)	5·2	3·9	40·5	10·8	3·3
(2) Rabbits previously given salt, six experiments, six rabbits					
Urine flow (ml./min.)	0·21	0·17	0·56	0·25	0·06
Cl′ excretion (μequiv./min.)	35	32	88	46	16·8
(3) Control experiment, no salt, five experiments, five rabbits					
	Before control		After control		
Urine flow (ml./min.)	0·31	0·24	0·15	0·11	0·10
Cl′ excretion (μequiv./min.)	5·5	5·2	5·1	5·0	—

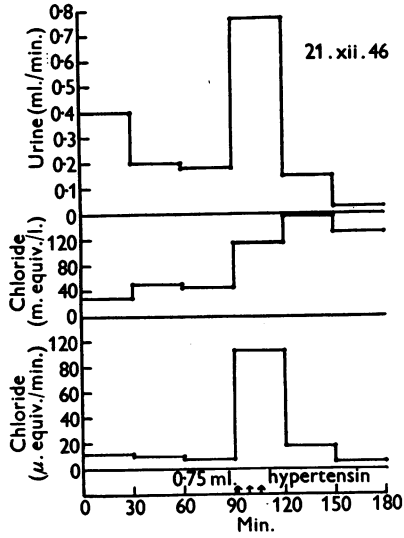

Fig. 2. Rabbit 478, 21 December 1946. Effect of three intravenous injections of 0·35 ml. hypertensin at 4 min. intervals on the rate of urine flow (top line), urinary chloride content (second line) and the urinary chloride excretion (bottom line) over the successive half-hour periods. The rabbit received 100 ml. H_2O by stomach tube 5 hr. before the figure begins.

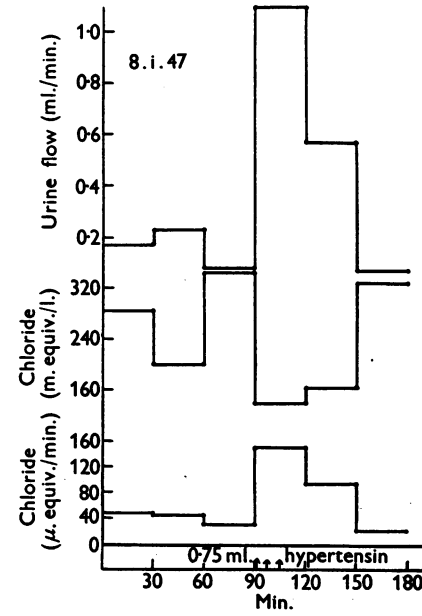

Fig. 3. Rabbit 486, 8 January 1947. Effect of hypertensin when chloride excretion was initially high. The rabbit had received 100 ml. of 1·5% solution of sodium chloride the evening before and again 5 hr. before the figure begins.

A similar effect on chloride excretion following the injection of renin had suggested that the phenomenon was due to an inhibition of chloride reabsorp-

tion with consequent increase in the excretion of water by osmotic action. This was tested and disproved by greatly raising the initial urinary chloride levels (Pickering & Prinzmetal, 1940). In this respect also, the action of hypertensin resembles that of renin. In order to raise the initial urinary chloride concentration, 100 ml. of 1·5% sodium chloride were given by stomach tube the evening before and again on the morning of the experiment, hypertensin being injected 4–5 hr. later. When the urinary chloride exceeded 180 m.equiv./l. hypertensin reduced the urinary chloride concentration, but produced the usual rise of urine output and of total chloride excretion (Table 1 and Fig. 3).

To ensure that these responses were due to hypertensin and not to an impurity present in our solutions, 'hypertensin control', prepared as previously described, was injected intravenously in similar amounts. This control preparation had no significant effect on the rabbit's blood pressure and, as Table 1 shows, was without influence on the rate of formation of urine, or its chloride content. It may be inferred, therefore, that the effects on the flow and chloride content of urine previously described were due to hypertensin itself.

As with renin, the diuretic effect seems to occur after the main pressor effect is over. In the case of renin, where the responses to a single injection are long drawn out, the initial phase of greatest rise of blood pressure was found associated with a reduced urine flow. Diuresis occurred later, often continuing, and sometimes being greatest, in the second half-hour when the arterial pressure had nearly or quite returned to normal. The response to hypertensin is briefer, and therefore more difficult to analyse in respect of time. We made no collections in periods shorter than 30 min. and never saw striking diuresis in the second half-hour period. Chloride excretion remained relatively constant over the separate half-hour collecting periods before hypertensin injection and changes were therefore more easily recognized. In rather more than half our experiments chloride excretion was still definitely raised in the second half-hour at a time, when, after similar doses, the rise of arterial pressure had subsided.

Comparison of hypertensin with tyramine and adrenaline. In a previous publication (Pickering & Prinzmetal, 1940) it was stated that tyramine acid phosphate produced no consistent increase in urine flow or chloride excretion. Renewed inspection of these data revealed a definite tendency to an increase in both these values, which was, however, trivial as compared with the effects produced by renin. It therefore seemed of some interest to compare the effects of hypertensin, tyramine and adrenaline, in doses having a similar pressor effect. The comparison was made on six rabbits using doses of 0·25 ml. hypertensin (preparation 465), 3 mg. tyramine acid phosphate (in 0·5 ml. saline) and 0·4 mg. adrenaline tartrate (in 0·4 ml.). These doses gave pressor responses of the same order. Thus the average response to hypertensin was 42 mm. and its duration 3½ min., to tyramine 46 mm. and 3½ min., and to adrenaline 46 mm.

and 1½ min. Two sets of experiments were done. In one the animals were hydrated with tap water; these rabbits had a low excretion of chloride. In the other, the animals were given 100 ml. of 1·5% sodium chloride by stomach tube the previous evening and deprived of water and food until the morning when they were again given 100 ml. of 1·5% sodium chloride instead of the usual hydrating dose of water; these animals had a high initial rate of chloride excretion. Each of the three substances was injected intravenously in three equal doses of the amounts mentioned at 3 min. intervals at the beginning of what is termed the first half-hour collecting period after administration. The results on urine flow are summarized in Table 2. Hypertensin produced an increase in

TABLE 2. A comparison of the effects of hypertensin, tyramine, and adrenaline on urine flow and chloride excretion

Substance injected	No. of rabbits	Urine flow (ml./min.) Before 1	Before 2	After 3	After 4	After 5	Chloride excretion (μequiv. Cl′/min.) Before 1	Before 2	After 3	After 4	After 5
After hydrating dose of 100 c.c. H_2O 5–7 hr. before											
Hypertensin	5	0·17	0·13	0·24	0·10	0·04	5·2	4·4	31·0	10·2	5·0
Tyramine	5	0·15	0·13	0·22	0·10	0·05	1·4	1·6	6·2	1·9	1·6
Adrenaline	3	0·27	0·24	0·29	0·17	0·12	8·0	8·0	12·4	—	—
After 100 c.c. 1·5% NaCl 5–7 and 21–23 hr. before											
Hypertensin	6	0·21	0·17	0·56	0·25	0·05	37	34	90	48	16·8
Tyramine	6	0·25	0·18	0·32	0·08	0·06	35	30	51	22	18
Adrenaline	3	0·16	0·10	0·52	0·11	0·06	35	22	65	27	14

urine flow in all eleven experiments, tyramine in nine out of eleven, and adrenaline in five out of six. When the urinary chloride was low, all three substances produced a rise both in the chloride percentage and in the absolute excretion in the urine, but this was most pronounced with hypertensin. When the chloride percentage was high, all three substances produced an increased excretion of chloride, with a tendency for the chloride percentage in the urine to fall. With minor exceptions the average figures presented in Table 2 do not reveal any pronounced difference between the action of the three substances, which, in these doses, have a diuretic and chlorouretic action. Detailed inspection of the results reveals, however, that there is a striking difference between them in the extent to which they influence the chloride content of the urine in the post-injection period. This is shown in Fig. 4, which compares the chloride percentages in the urine collected in the successive periods immediately before and after injection of the substances. It will be seen that while in each case there is a tendency for the chloride content of the urine to approximate to a mean position, this tendency is feeble in the case of tyramine and adrenaline, but pronounced after hypertensin. Data from experiments previously reported (Pickering & Prinzmetal, 1940) show that in this respect hypertensin closely

resembles renin. In fact during the diuresis following the intravenous injection of renin or hypertensin, the concentration of chloride in the urine approximates to a value rather greater than that of plasma, and this occurs whether the urinary chloride was initially much below or much above that level.

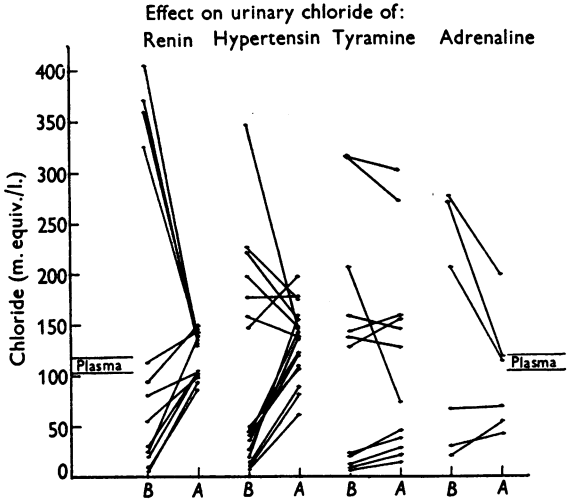

Fig. 4. Effect of renin, hypertensin, tyramine and adrenaline on the urinary chloride. Each line represents the change in urinary chloride from the period before (*B*) to the period after (*A*) the administration of the substance. The data for hypertensin, tyramine and adrenaline are from the experiments summarized in Table 2 (plus three additional experiments with the same dose of hypertensin), and the data for renin those obtained in a previous paper (Pickering & Prinzmetal, 1940).

The effect of renin and hypertensin on inulin and diodone clearances. The qualitative similarity between the effects of hypertensin, tyramine and adrenaline on the volume and composition of the urine in the unanaesthetized rabbit suggest that these effects may be vascular in origin. To investigate this further we have measured the renal clearances of inulin and diodone over half-hour periods before, immediately after, and an hour or more after the intravenous injection of renin and hypertensin. The results are summarized in Table 3 which includes data for the periods only in which these clearances were measured, the urine flows and chloride contents for the other periods being omitted.

Five experiments were done in which a single dose of renin was injected at the beginning of a collecting period. In all, a diuresis and a conspicuous increase in the urinary excretion of chloride occurred, and in the two experiments where this was measured a concomitant increase in the excretion of sodium. In two experiments the increase in urine volume and chloride excretion was greatest in the second half-hour, and this was true in two other similar observa-

TABLE 3. The effect of renin and hypertensin on inulin and diodone clearances in the rabbit

Date and rabbit	Period	Urine flow (ml./min.)	C_{IN} (ml./min.)	C_D	F.F. (%)	Urine (μequiv./min.) Na	Urine (μequiv./min.) Cl	Urine (m.equiv./l.) Na	Urine (m.equiv./l.) Cl	Serum (m.equiv./l.) Na	Serum (m.equiv./l.) Cl
24. ix. 46	2.47–3.17	0·12	5·0	30·9	16·1	1·15	2·8	9·6	23·3	150	—
478	3.20	3·0 ml. (7·5 mg.) renin									
	3.17–3.45	0·25	5·4	21·5	25·1	23·5	27·0	94·0	108·0	149	—
	3.45–4.16	0·08	5·3	26·8	19·7	10·2	12·6	127·0	156·3	148	—
26. ix. 46	1.52–2.22	0·081	6·2	35·5	17·4	2·8	8·8	33·0	109·0	140	108
479	2.25	3·0 ml. (9 mg.) renin									
	2.22–2.52	0·216	7·1	27·6	25·7	16·8	27·6	78·0	128·0	144	106
	2.52–3.22	0·37	7·1	36·3	19·6	44·4	52·2	120·0	141·0	140	105·5
	4.24–4.58	0·10	7·3	—	—	—	5·7	—	57·0	—	105
26. viii. 46	2.00–2.30	0·38	6·4	32·2	19·9	—	6·0	—	15·8	—	—
472	2.40	3 ml. (9 mg.) renin									
	2.30–3.00	0·26	4·9	16·6	29·5	—	22·7	—	87·2	—	—
	3.00–3.30	0·43	5·7	23·4	24·4	—	67·5	—	157	—	—
	3.30–4.00	0·03	4·1	19·0	21·6	—	5·4	—	179	—	—
29. viii. 46	3.30–4.00	0·017	8·0	34·7	23·0	—	3·0	—	176	—	—
477	4.04	4 ml. (12 mg.) renin									
	4.00–4.30	0·30	6·7	21·1	31·8	—	33·0	—	110	—	—
	5.00–5.30	0·033	6·0	31·8	18·9	—	1·61	—	48·7	—	—
4. ix. 46	3.30–4.02	0·033	5·8	31·8	18·3	—	1·5	—	45·6	—	—
478	4.04	2 ml. (6 mg.) renin									
	4.02–43.2	0·42	5·8	19·5	29·8	—	50·4	—	120	—	—
	5.00–53.0	0·017	6·1	34·0	18·0	—	0·27	—	15·7	—	—

1. viii. 46	2.50–3.20	0·27	8·1	—	—	9·7	14·7	36	54·5	139	104
474	3.52	3 ml. hypertensin (1 mm.–40 mm. Hg)									
	3.52–4.25	0·755	7·1	—	—	68·2	60·5	90	80	142	106·7
	4.58–5.25	0·036	6·0	—	—	2·4	2·62	66	73	142	108·6
2. x. 46	3.03–3.35	0·20	7·7	42·1	18·3	1·38	5·4	6·9	27	144	—
479	3.37–3.49	1·5 ml,. 1·0 ml., 1·0 ml. hypertensin									
	3.35–4.06	0·20	7·8	30·4	25·7	19·8	28·4	99	142	147	—
	4.34–5.07	0·09	7·1	33·8	21·0	6·6	11·4	74	127	—	—
12. viii. 46	3.10–3.39	0·53	7·0	34·5	20·3	—	8·0	—	15	—	113
472	4.11	2·5 ml. hypertensin									
	4.08–4.38	0·65	8·0	27·1	29·5	—	52·0	—	80	—	112
	5.08–5.38	0·033	7·4	34·6	21·4	—	2·3	—	69·6	—	114
13. viii. 46	3.40–4.10	0·116	7·8	40·2	19·4	—	3·25	—	28	—	—
473	4.10–4.17	1·0 ml. 1·5 ml. 1·0 ml. hypertensin									
	4.10–4.40	0·05	6·3	26·7	23·6	—	4·5	—	90	—	—
	5.10–5.40	0·033	8·4	44·0	19·1	—	1·65	—	50	—	—
13. viii. 46	3.45–4.15	0·127	8·4	45·7	18·4	—	2·69	—	21·2	—	108
475	4.49–4.58	1·5 ml. 1·5 ml. and 1·5 ml. hypertensin									
	4.48–5.15	0·52	3·8	13·0	29·2	—	76·4	—	147	—	124
	5.15–5.45	0·95	4·9	16·2	30·2	—	144·0	—	152	—	—
	5.45–6.15	0·15	3·1	16·4	18·9	—	24·0	—	160	—	115
2. x. 46	3.00–3.31	0·14	6·0	34·6	17·3	1·37	5·1	9·8	36·2	147	—
478	4.02–4.18	1·0 ml. 1·5 ml. and 1·0 ml. hypertensin control									
	4.00–4.31	0·12	6·6	37·3	17·7	2·04	6·4	17	53·2	147	—
	4.31–5.03	0·039	6·2	30·0	20·7	2·24	6·2	57·5	158	145	—

tions on one of these rabbits, 479, in which clearances were not measured. Renin produced no constant or striking change in inulin clearance measured over the half-hour period, a result confirming that earlier obtained for creatinine clearance (Pickering & Prinzmetal, 1940). Diodone clearance was always reduced significantly in the first half-hour period after renin, and the filtration fraction was conspicuously raised. In rabbit 479, diodone clearance and filtration fraction had returned to normal in the second half-hour period in which the increase in urinary volume and chloride was most pronounced (Fig. 5). In rabbit 472, however, in which the greatest increase in urinary volume and

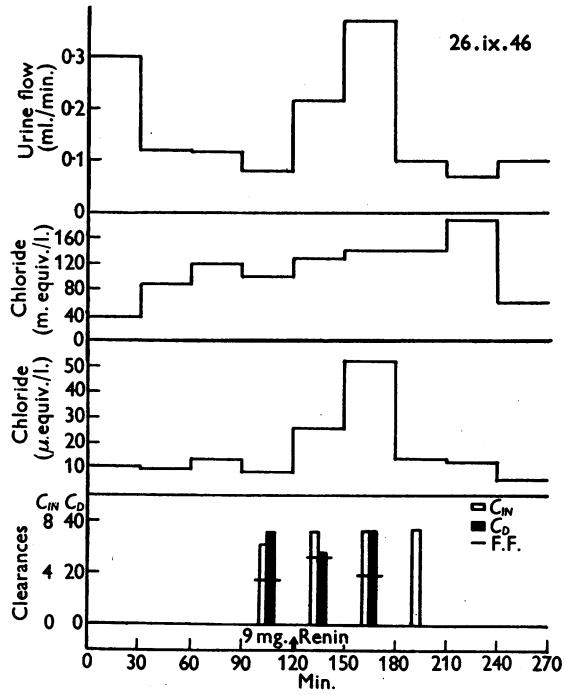

Fig. 5. Effect of 3 ml. renin on urine flow, chloride excretion, inulin and diodone clearances and the filtration fraction. Rabbit (479) unanaesthetized. In this and the two subsequent figures the scale for filtration fraction (*FF*) is that for diodone clearance (*CD*).

chloride also occurred in the second half-hour, diodone clearance and filtration fraction had not returned to their pre-injection level. In the third half-hour both inulin and diodone clearances were low, though the filtration fraction was normal, a result which may have been due to incomplete collection of urine, though it seems from our data that this rarely occurred.

The effects of hypertensin on the clearances were in general similar to those of renin. During the collecting period at the beginning of which the injections were given, diodone clearance always fell significantly and the filtration fraction was increased. Changes in inulin clearance were variable and, with one

exception, slight. An hour after the injection inulin and diodone clearances and filtration fraction had returned to normal or nearly so in all experiments except one. The exceptional rabbit (475) responded with a very large diuresis, particularly in the second half-hour, the urinary chlorides rising to above plasma level in both periods, and it developed signs of acute pulmonary oedema after the third injection of hypertensin. Inulin clearance was significantly reduced during both periods of large urinary outputs and remained low during subsequent half-hour collecting periods in which the diuresis and the increased chloride excretion had subsided (Fig. 6). So far as we could ascertain this response was due to hypertensin, because subsequent injections of smaller

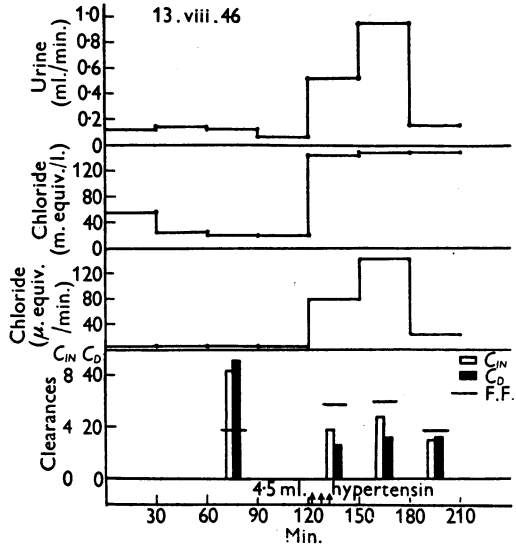

Fig. 6. Very intense diuresis to hypertensin with pronounced effects on urinary chloride, and the clearances. Rabbit (475) unanaesthetized.

doses led to no abnormal symptoms, and the control preparation injected subsequently in similar amounts was without effect on urinary chloride or output. The effects on clearances and filtration fraction in the rabbit are similar to those which others have reported with both renin and hypertensin in man and the dog, though in neither species has a similar effect on urinary chloride and output been described.

With renin, difficulty had been experienced in obtaining satisfactory diureses when the interference inherent in blood sampling was added. To ensure a diuretic response it proved to be necessary to obtain blood samples from an ear previously desensitized by nerve section (Pickering & Prinzmetal, 1940). It was also found with renin that the diureses obtained under such conditions were less

pronounced than when no blood samples were taken. Even when the ear is insensitive, blood sampling, necessitating as it does the animal being restrained for 3 or 4 min. while its ear is manipulated, produces a change in the experimental conditions. It is to this that we attribute the failure of diuresis to occur during the period at the beginning of which hypertensin was injected in rabbits 473 and 479. Each of these animals did, however, show the usual changes in urinary chloride, large in 479 (Fig. 7), small in 473.

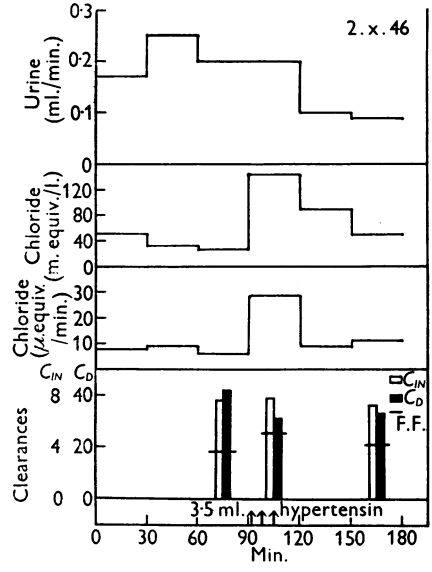

Fig. 7. Effect of hypertensin on glomerular filtration rate, diodone clearance, filtration rate, and chloride excretion; no diuresis. Rabbit (479) unanaesthetized.

The action of renin on the tubular reabsorption of glucose. A rise in the effective head of pressure in the glomeruli leads to an increased excretion of water and salt in both the isolated kidney (Richards & Plant, 1922; Starling & Verney, 1925) and anaesthetized animal (Eggleton, Pappenheimer & Winton, 1940), presumably because of the more rapid flow of urine along the tubules. The effects of renin and hypertensin might well be due to such a mechanism for both substances raise arterial pressure, and the fraction of plasma filtered in traversing the glomeruli. If the number of functioning nephrons remained unchanged, an increased flow along the tubules could only be achieved by a greatly increased glomerular filtration rate. But the inulin clearances show that the glomerular filtration rate is not necessarily increased when urine flow and urinary chloride are raised by hypertensin or renin. It seems, therefore, that if we are dealing with a pressure diuresis it must be one in which a reduced number of nephrons participate. To see whether this were so, we determined the capacity of the tubules to reabsorb glucose (glucose Tm, Smith, 1939) at blood glucose values above 600 mg./100 c.c., using creatinine clearance as a measure of glomerular filtration rate. These experiments were performed under nembutal anaesthesia to allow the maintenance of adequate plasma glucose and creatinine levels by intravenous infusion of the substances. Fig. 8 illustrates and Table 4 summarizes these experiments in which a single injection of two units of renin was given. After renin, urinary output rose above the pre-injection level in two experiments out of five in the first 10 min. and in five out of six in the second 10 min. Chloride excretion was increased in both first and second 10 min. periods in all four experiments where it was measured and particularly in the second 10 min.

TABLE 4. Effect of renin on creatinine clearance and tubular capacity to reabsorb glucose (glucose Tm)

(Successive 10 min. clearance periods are indicated by −3, −2, −1 before and +1, +2, +3 after injection of renin.)

		Urine flow (ml./min.)						Chloride excretion (μequiv./min.)					Creatinine clearance (ml./min.)				Glucose Tm (mg./min.)			
		Before renin			After renin			Before renin		After renin			Before renin		After renin		Before renin		After renin	
Rabbit	Date	−3	−2	−1	+1	+2	+3	−2	−1	+1	+2	+3	−2	−1	+1	+2	−2	−1	+1	+2
494	29. x. 47	0·13	0·21	0·4	—	0·95	1·35	—	—	—	—	—	10·1	11·3	—	9·2	29·9	33·5	—	31·7
487	25. x. 47	—	1·3	1·25	1·3	3·2	1·95	—	—	—	—	—	7·9	8·3	8·8	10·3	20·8	28·0	32·1	29·2
487	30. x. 47	—	0·73	0·68	1·36	2·4	2·16	30·5	13·5	61·0	204	140	6·4	6·9	7·9	8·2	20·6	22·0	23·7	24·3
486	5. xi. 47	0·95	0·75	0·88	0·76	1·76	1·21	33·0	32·0	37·0	104	64·0	6·7	7·4	6·6	8·9	25·3	29·4	30·1	36·9
494	6. xi. 47	2·14	1·45	1·58	1·32	1·33	0·65	11·0	4·47	57·8	73·0	73·6	11·7	13·4	12·4	10·2	29·1	36·5	35·8	29·1
486	13. xi. 47	0·27	0·2	0·24	0·41	0·91	—	29·1	19·0	56·5	69·9	—	6·5	6·7	6·5	6·9	18·4	21·0	22·1	25·9

TABLE 5. Effect of renin and hypertensin on sodium and chloride reabsorption

Exp. and date	Period	C_{IN} (ml./min.)	Urine flow (ml./min.)	Water (% excreted)	Chloride: Glom. filtrate (μmol./min.)	Chloride: Excreted (μmol./min.)	Chloride: % excreted	Sodium: Glom. filtrate (μmol./min.)	Sodium: Excreted (μmol./min.)	Sodium: % excreted
1	2.47–3.17	5·0	0·12	2·4	—	—	—	750	1·15	0·15
24. ix. 46	3.20	7·5 mg. renin in 3 ml.								
	3.17–3.45	5·4	0·25	4·6	—	—	—	850	23·5	2·9
	3.45–4.16	5·3	0·08	1·5	—	—	—	785	10·2	1·3
2	1.52–2.22	6·2	0·081	1·3	670	8·8	1·3	868	2·7	0·32
26. ix. 46	2.25	9 mg. renin in 3 ml.								
	2.22–2.52	7·1	0·216	3·0	753	27·6	3·7	1022	16·8	1·6
	2.52–3.22	7·1	0·37	5·2	748	52·2	7·0	994	44·4	4·5
	4.24–4.58	7·3	0·10	1·4	766	5·7	0·74	—	—	—
6	2.50–3.20	8·1	0·27	3·3	842	14·7	1·7	1126	9·7	0·86
1. viii. 46	3.52	3 ml. hypertensin								
	3.52–4.25	7·1	0·755	10·6	756	60·5	8·0	1008	68·2	6·8
	4.58–5.25	6·0	0·036	0·6	651	2·62	0·4	852	2·4	0·28
7	3.03–3.35	7·7	0·20	2·6	—	—	—	1109	1·38	0·12
2. x. 46	3.37–3.49	3·5 ml. hypertensin								
	3.35–4.06	7·8	0·20	2·6	—	—	—	1145	19·8	1·7
	4.34–5.07	7·1	0·09	1·3	—	—	—	1029	6·66	0·64
8	3.10–3.39	7·0	0·53	7·6	791	8·0	1·0	—	—	—
12. viii. 46	4.11	2·5 ml. hypertensin								
	4.08–4.38	8·0	0·65	8·1	896	52·0	5·8	—	—	—
	5.08–5.38	7·4	0·033	0·45	844	2·3	0·27	—	—	—
10	3.45–4.15	8·4	0·127	1·51	907	2·69	0·30	—	—	—
13. viii. 46	4.49–4.58	4·5 ml. hypertensin								
	4.48–5.15	3·8	0·52	13·7	471	76·4	16·2	—	—	—
	5.15–5.45	4·9	0·95	19·4	—	—	—	—	—	—
	5.45–6.15	3·1	0·15	4·8	356	24·6	6·75	—	—	—
11	3.00–3.31	6·0	0·14	2·3	—	—	—	882	1·37	0·16
2. x. 46	4.02–4.18	3·5 ml. hypertensin control								
	4.00–4.31	6·6	0·12	1·8	—	—	—	970	2·04	0·21
	4.31–5.03	6·2	0·039	0·63	—	—	—	899	2·24	0·25

The figures for sodium and chloride in the glomerular filtrate have been obtained by multiplying plasma Na and Cl by inulin clearance. No allowance has been made for Donnan equilibrium or for the space occupied by the plasma proteins.

The increased output of urine and of chloride effected by renin are not accompanied by any constant change in glomerular filtration rate or glucose Tm.

Appraisal of these results is complicated by the tendency, least marked in Fig. 8, of urinary output to rise during the period before injection of renin, a tendency no doubt resulting from the continuous intravenous infusion. As Kaplan & Smith (1935) and Dicker & Heller (1944–5) have shown, the rabbit is peculiar in that more nephrons are called into action as urinary output rises in water diuresis. Both creatinine clearance and glucose Tm were rising in the period immediately preceding renin injection, and we cannot exclude the possibility that any further rises in the periods following renin injection were

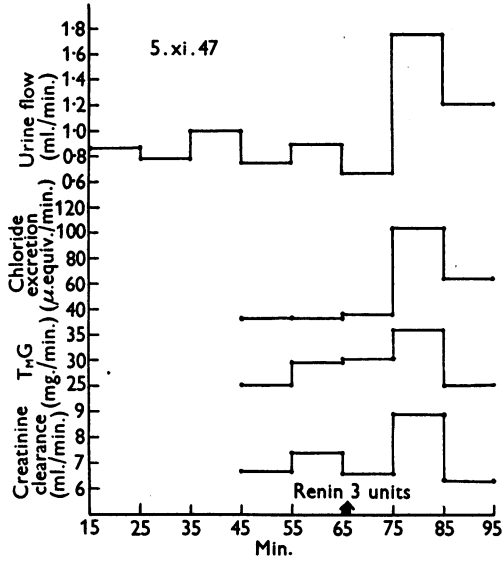

Fig. 8. Effect of renin on urine flow, chloride excretion, glucose Tm, and creatinine clearance in a rabbit anaesthetized with nembutal. Urine collections at 10 min. intervals.

due to a continuation of this response to increasing water load. There is, however, no difficulty in deciding from these figures whether or not renin diuresis is due to an increased rate of glomerular filtration per functioning nephron, which can be gauged by the ratio $\frac{\text{creatinine clearance}}{\text{glucose Tm}}$. Taking the two experiments (Exps. 2 and 4) with the largest diuretic response to renin, this ratio was in the two periods before renin 0·37 and 0·30, and in the two periods after renin 0·27 and 0·35 in Exp. 2. In Exp. 4, the corresponding figures were 0·26, 0·25, 0·22, 0·24. The ratio remained comparatively steady throughout each experiment, and was at its height before the renin response in four of the six experiments. Thus, although we may not legitimately infer from these experiments that renin

has any definite effect on glucose Tm, we are justified in concluding that the striking increase in chloride excretion and change in the chloride content of the urine are not due to an increased flow of glomerular fluid down the individual nephrons. They are clearly due to a change in activity of the tubular cells.

DISCUSSION

The experiments here described show that hypertensin has an effect on the rabbit's kidney that is qualitatively identical with that of renin. In adequate doses both increase urinary output, both increase sodium and chloride excretion, both affect the inulin and diodone clearances in the same way, both produce a urine whose chloride content tends to approximate and slightly to exceed that of plasma. We have not been able to make a quantitative comparison between the actions of the two substances, because of the difficulty of securing a constant renal response to a given dose and because the effects of the two substances differ so much in duration. Nevertheless, our results, while offering no proof, are at least consistent with the hypothesis that the action of renin on the kidney, as on the vessels, is mediated by hypertensin.

It has been already shown (Pickering & Prinzmetal, 1940) that the diuresis produced by renin is not a simple consequence of increased glomerular filtration rate. This paper affirms that conclusion, and admits a similar statement for hypertensin. The changes in excretion of water, sodium and chloride are evidently of tubular origin. It now seems most improbable that a pressor diuresis is concerned. For urine flow and urinary chloride changes may be near their height when arterial pressure has regained the normal range (Pickering & Prinzmetal, 1940), and when the change in the conditions of glomerular filtration as revealed by the filtration fraction has subsided. Again, no evidence has been obtained of any increase in the volume of fluid presented by a functioning glomerulus to its corresponding tubule.

In the case of renin, evidence has been adduced that tubular reabsorption of water, of sodium and of chloride are diminished (Pickering & Prinzmetal, 1940). The observations recorded in this paper have revealed that hypertensin exerts a similar action. A measure of these changes may be found in Table 5 which shows the percentages of water, sodium and chloride entering the tubules that are finally excreted and the effects on these percentages of renin, hypertensin and hypertensin control. It will be seen that renin and hypertensin both diminish tubular reabsorption of water, chloride and sodium, while 'hypertensin control' is without demonstrable effect on water or sodium reabsorption. Moreover, the reabsorption of water, of sodium and of chloride is more or less equally suppressed by renin or hypertensin. These measurements, together with the demonstration that both renin and hypertensin produce a urine which approximates in chloride content to that of plasma, indicate that

the chief effect of these substances is to suppress the differential reabsorption of water and chloride in the tubules.

In recent years much has been learned of the division of function within the nephron by the beautiful techniques developed by Richards and his school for obtaining fluid from the glomerular capsule and from different parts of the tubule. In both amphibian and mammal the glomerular filtrate may be accepted as an ultrafiltrate of plasma. Walker, Bott, Oliver & McDowell (1941) have shown that glucose is completely reabsorbed in the first half of the proximal tubule in rat and guinea-pig, and that in this part of the tubule water is absorbed at the rate of 12·5% of the fluid entering the tubules in rats and 7·5% in guinea-pigs per 10% of the total length of the proximal tubule. They were unable to collect fluid from the distal half of the proximal tubules, but, accepting a similar rate of water absorption, estimated that about 80% of the water presented to the tubules was absorbed in the proximal convolution. In amphibia, Walker, Hudson, Findley & Richards (1937) had found that the chloride content of the proximal tubule was higher than that of plasma, even when allowance had been made for protein differences, but had been unable to exclude a technical error. In the guinea-pig and rat the proximal tubule fluid was found consistently richer in chloride than plasma: 'the fluid plasma concentration ratio reaches an average of 1·40 in the first third of the proximal tubule and remains at this point without further increase throughout the second third' (Walker *et al.* 1941). The osmotic pressure of the proximal tubule was found to be identical with that of plasma, and Walker and his colleagues therefore suspected that the increased concentration of chloride was balanced by a fall in the concentration of some other ion. Beyond the proximal tubule little is known of division of function in the mammalian nephron. It has been inferred from comparative anatomy that the loop of Henle is the chief site of water absorption (Burgess, Harvey & Marshall, 1933), and that it is here in particular that the hormone of the posterior pituitary acts. Walker *et al.* (1941) measured in three rats the osmotic pressure of fluid obtained from the beginning of the distal tubule and found it actually significantly less than that of plasma. They suggested, therefore, that chloride absorption took place proximally to reabsorption of water, and possibly in the loop of Henle. Thus it would seem that in the diuretic phase the chief action of renin and hypertensin on the kidney of the rabbit, that of suppressing the differential reabsorption of water and salt, is located in the tubules distal to the proximal convolution. Although, as has previously been shown, the ratio urinary chloride to plasma chloride after renin tends to be greater than 1, the ratio has not yet been observed as high as 1·4, the value characteristic of proximal tubular urine in the mammal; it seems probable that the observed differences in chloride content of urine and plasma after renin can be accounted for by differences in protein content of the two fluids and the Donnan equilibrium.

It is by no means impossible that the tubular action of renin and hypertensin has a vascular basis. It is, for example, possible that blood is diverted from that part of the tubule concerned in differential water and chloride reabsorption, a possibility we have not been able to test experimentally. Peter (1909) and Huber (1909–10) have shown that there is a striking contrast between the nephrons whose glomeruli are situated in the periphery of the cortex and those originating in the juxta-medullary zone; the former have short loops of Henle, largely situated in the cortex, the latter long loops which plunge deeply into the medulla. It has been suggested that the urine formed by these two types of nephron is different (Trueta, Barclay, Daniel, Franklin & Prichard, 1947). The possibility arose that renin might divert blood from one type to the other. Following the injection of Berlin blue as previously described it was possible to demonstrate the interlobular arteries, their afferent glomerular branches and the glomerular tufts. We observed no consistent difference from the normal in the proportion of glomeruli filled nor in the distribution of these glomeruli in the kidneys obtained at the height of renin diuresis. We do not think, therefore, that the change in the quantity and quality of the urine secreted after renin can be due to diversion of blood from one type of nephron to another, but we cannot exclude a diversion of blood away from some part of the tubules.

We wish to thank Dr A. E. Barclay and Miss M. L. Prichard for advice about injecting the renal vessels.

SUMMARY

1. In the unanaesthetized rabbit, hypertensin increases the rate of urine flow, and conspicuously increases chloride excretion. Urinary chloride concentration, whether high or low initially, tends to approximate to that of plasma.

2. Adrenaline and tyramine, in doses giving similar rises of arterial pressure, act in a manner qualitatively similar to hypertensin, but with a much less conspicuous effect on urinary chloride.

3. In the doses used, hypertensin has no constant effect on inulin clearance, but always reduces diodone clearance and increases the filtration fraction. The effect of renin is similar.

4. In the anaesthetized rabbit renin has no conspicuous effect on glucose Tm during the period of diuresis.

5. The evidence is compatible with the view that the effect of renin on the kidney is mediated by hypertensin.

6. The results suggest that the changes in the volume and composition of the urine during the diuresis produced by renin and hypertensin are due to suppression of the tubular capacity differentially to reabsorb water, sodium and chloride.

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