J. Physiol. (1969), **201**, *pp.* 349–368
With 6 text-figures
Printed in Great Britain

THE ROLE OF A RENAL THIRST FACTOR IN DRINKING INDUCED BY EXTRACELLULAR STIMULI

BY J. T. FITZSIMONS

From the Physiological Laboratory, University of Cambridge, England

(*Received* 16 *August* 1968)

SUMMARY

1. Rats in normal fluid balance drank water 1–2 hr after complete ligation of the inferior vena cava either above or below the renal veins. At the same time there was a fall in urine flow and excretion of electrolyte, especially after caval ligation above the renal veins, so that the animals ended the initial 6 hr period in positive fluid balance.

2. Caval ligation was relatively ineffective as a stimulus to drinking after bilateral nephrectomy, but was effective in rats made anuric by ureteric ligation.

3. Rats subjected to caval ligation and offered a choice between water and 1·8 % saline (w/v) drank water, despite the increasing hypotonicity of the body fluids thereby resulting.

4. During the secondary polydipsia, which generally occurred on about the third day after caval ligation as renal function was recovering, there was an increased preference for 1·8 % saline.

5. Constriction of the aorta above the renal arteries, or constriction of both renal arteries, also caused drinking, oliguria and the development of positive fluid balance.

6. Constriction of the aorta below the renal arteries, or after nephrectomy, was ineffective as a stimulus to drinking.

7. Saline extracts of renal cortex caused rats in normal water balance to drink. Activity was destroyed by boiling the extract for 10 min. Renal medullary and hepatic extracts were without effect on drinking.

8. It proved impossible to separate dipsogenic and pressor activities of renal extracts during the different stages of fractionation which lead to the production of renin; disappearance of one activity was invariably accompanied by disappearance of the other.

9. Dipsogenic and pressor actions were greater in nephrectomized rats than in normal rats.

10. Both extractable dipsogenic factor and extractable pressor activity were reduced by treating the rat with DOCA and saline for several weeks beforehand.

11. The renal dipsogen therefore has similar properties to renin. It may prove to be identical with renin, particularly in view of the fact that angiotensin also stimulates drinking.

12. Adrenalectomy did not affect drinking induced by renin or by caval ligation.

13. It is concluded that the renin angiotensin system may play a role in the genesis of the thirst which follows certain extracellular stimuli.

INTRODUCTION

A number of procedures which deplete the extracellular space without affecting the cellular space have been shown to cause drinking in the rat. These include Na depletion by dietary means (Swanson, Timson & Frazier, 1935) or by peritoneal dialysis (Semple, 1952; Falk, 1961); isotonic depletion by hyperoncotic peritoneal dialysis (Fitzsimons, 1961) or hyperoncotic subcutaneous dialysis (Stricker, 1966); and haemorrhage (Fitzsimons, 1961; Oatley, 1964).

A feature common to these procedures is that they all adversely affect the circulation of the blood by causing actual or incipient hypovolaemia. In view of this and the fact that blood vessels all over the body are abundantly endowed with possible receptors it was reasonable to expect that procedures which alter the circulation but which do not immediately affect fluid balance might also cause drinking. This expectation was confirmed. Complete ligation of the abdominal inferior vena cava in the rat caused osmotically inappropriate drinking, but unexpectedly, it proved much less effective as a stimulus of drinking in the nephrectomized rat (Fitzsimons, 1964).

The nature of this renal contribution to drinking is further examined here and evidence is presented which suggests that certain stimuli of drinking produce their effects on water intake by causing secretion of a renal thirst factor, probably renin. Some of the experiments described here have been reported to the Physiological Society (Fitzsimons, 1966, 1967).

METHODS

A mixed population of male albino rats weighing between 150 and 400 g were used in these experiments. For several days before the drinking tests the animals were kept in standard rat cages in the room where the experiments were performed. During this time care was taken to ensure that the rats did not run short of food or water. Rats were handled frequently and any which appeared unduly upset by handling were not used in these experiments.

Metabolism cages. During the drinking tests the rats were confined to individual metabolism cages constructed of wire netting. The cages were placed over funnels with

stainless-steel sieves to separate faeces and urine, and the urine was collected in graduated centrifuge tubes. Water was available from a manometric device which enabled a continuous record of drinking to be taken. Food was not available.

In some long-term preference experiments the animals were confined to individual metabolism cages for many days. During this time the rats had access to water and 1·8% saline. Powdered rat diet was also available from non-spill containers except during the standard 6 hr drinking test.

Surgical procedures. All surgical procedures were carried out under open ether anaesthesia using a clean surgical technique.

Caval ligation: the inferior vena cava was approached through a small muscle-splitting incision in the right loin and was completely ligated, either above the renal veins and below the hepatic and right adrenal veins, or immediately below the renal veins. This procedure is facilitated if the right kidney is used as a retractor. In other experiments caval ligation was performed immediately after bilateral nephrectomy, or bilateral ureteric ligation, or bilateral adrenalectomy.

Constriction of the abdominal aorta: the aorta was approached through a small muscle-splitting incision in the left loin. It was mobilized and a ligature placed in position around it just above the renal arteries. Included in the ligature was a wire of diameter 0·25–0·5 mm. The ligature was tightened and the wire removed thus allowing flow of blood to continue. In one group of controls ligatures were placed in a similar manner below the renal arteries; in another group bilateral nephrectomy was performed at the same operation as aortic constriction.

Preparation and assay of renal extracts. Rat and pig renin were prepared by the method, slightly modified, of Haas, Lamfrom & Goldblatt (1954). The details of the extraction procedure are as follows. The kidney was decapsulated, the pelvis and perinephric fat were removed and the organ was then cut into small pieces and extracted with distilled water (1 ml./g tissue) in a tissue homogenizer. The tissue debris was removed by centrifuging. The supernatant was heated to 55° C for 10 min and then acidified with 1 N-HCl to pH 1·6 for a further 10 min after which the pH was brought back to about 7 by the slow addition of 2 N-NaOH. The denatured protein was removed by centrifuging and renin was precipitated from the supernatant by adding sufficient solid $(NH_4)_2SO_4$ to make 2·5 M-$(NH_4)_2SO_4$ (0·33 g $(NH_4)_2SO_4$/ml.). After some hours the precipitated renin was removed and dissolved in water. It was then dialysed in Visking cellulose sacs against several changes of distilled water over a period of about 48 hr. The volume of renin solutions was adjusted so that 1 ml. of solution contained the renin from 1 g of fresh kidney.

At various stages of the fractionation procedure (see Fig. 3) extracts were assayed for dipsogenic and pressor activities using the activities of a simple saline extract of kidney as the standards of reference. Dipsogenic activity was tested by measuring the amounts of water drunk in 6 hr by groups of nephrectomized rats given either the test or the standard extract by intraperitoneal injection. Pressor activity was assayed by measuring the rise in carotid blood pressure of the anaesthetized, ganglion blockaded (Pentolinium tartrate, Ansolysen retard 2·5 mg/100 g, S.C.) rat given aliquots of the same extracts by intravenous injection through a jugular catheter.

Experimental procedure and calculation of results. Immediately after the surgical procedures or injections the animals were weighed to the nearest 0·1 g and placed in individual metabolism cages where they were free to drink but not eat for the next 6 hr. Where appropriate, urine was measured and analysed for Na and K by flame photometry. At the end of the experimental period the rats were again weighed to 0·1 g and the amounts of water drunk was measured.

In some experiments, samples of blood were taken by cutting off the tip of the tail and collecting the blood which was then analysed for Na and K. Depression of freezing points were also measured on these samples.

Except where otherwise stated all results are expressed per 100 g of rat.

RESULTS

Caval ligation as a stimulus to drinking. Complete ligation of the abdominal inferior vena cava, either above the renal veins and below the hepatic and right adrenal veins, or immediately below the renal veins, was well tolerated by the male albino rat. The infrahepatic vena cava carries about one-third of the venous return to the heart so that the immediate effect of caval ligation was a sharp diminution in cardiac

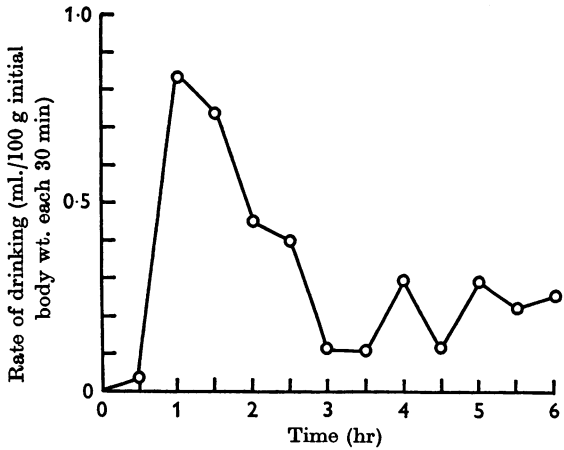

Fig. 1. The rate of drinking in the 6 hr following caval ligation above the renal veins. Each point is the mean of ten observations.

output. Mean arterial blood pressure fell to about half its previous value and then started to recover within a few minutes of ligation, though it remained lower than normal for several hours afterwards. Ascites did not occur, in contrast to the considerable abdominal fluid retention which may occur after suprahepatic caval ligation, though occasionally there resulted some retroperitoneal oedema localized to the region just below the ligature. When a caval ligature placed at a preliminary operation was pulled tight in a conscious animal there appeared to be little disturbance save an awareness by the rat that something was happening. Consciousness was fully maintained. Caval ligation above the renal veins caused considerable venous engorgement of the kidneys; infrarenal caval ligation did not have this effect.

The rat was usually recovering consciousness by the time it was placed in the metabolism cage. About $\frac{1}{2}$–1 hr later it started to drink and it continued drinking for an hour or two after this (Table 1; Fig. 1).

After caval ligation above the renal veins there was also a marked fall in urine flow and in the rate of electrolyte excretion. A consequence of

TABLE 1. Drinking and excretion in the 6 hr which followed caval ligation, sham operation, or caval ligation with adrenalectomy

	Water drunk ml./100 g initial body wt.	Change in body wt. g/100 g initial body wt.	Urine volume ml./100 g initial body wt.	Na μM/100 g initial body wt.	K μM/100 g initial body wt.
Caval ligation above renal veins (17)	4·20 ± 0·24	+ 2·77 ± 0·63	0·24 ± 0·08	16 ± 4·7	17 ± 8·5
Caval ligation below renal veins (10)	4·17 ± 0·97	+ 0·48 ± 0·67	2·04 ± 0·22	19 ± 5·4	124 ± 23·2
Sham caval ligation (10)	0·43 ± 0·08	− 2·10 ± 0·39	0·86 ± 0·10	47 ± 7·8	187 ± 15·7
Adrenalectomy and caval ligation above renal veins (10)	4·25 ± 0·60	+ 3·07 ± 0·59	0·08 ± 0·03	6 ± 3·0	9 ± 6·9

In this and subsequent tables, mean value ± S.E. of mean with the number of observations in parentheses is given.

drinking coupled with extreme oliguria was that the rat developed a substantial positive fluid balance at the end of the initial drinking period of 6 hr.

Rats adrenalectomized just before being subjected to caval ligation showed an increase in water intake similar to that following caval ligation in the normal rat (Table 1).

The serum osmotic pressure and serum Na tended to fall while the animal was drinking, despite the oliguria, and serum K rose. In five experiments the mean values for serum Na and K were 147·4 and 5·3 m-equiv/l. immediately after caval ligation; 6 hr later the values were 139·4 and 5·9 m-equiv/l. respectively. The drinking which followed caval

TABLE 2. Amounts of water and 1·8% saline drunk in the first 6 hr after caval ligation above the renal veins

	Water drunk ml./100 g initial body wt.	1·8% saline drunk ml./100 g initial body wt.	Change in body wt. g/100 g initial body wt.
Caval ligation above renal veins (8)	3·69 ± 0·50	0·11 ± 0·03	+3·16 ± 0·74
Sham caval ligation (8)	0·40 ± 0·08	0·12 ± 0·04	−1·70 ± 0·14

ligation above the renal veins was therefore osmotically inappropriate since it led to dilution of the body fluids. Nevertheless, rats which had been allowed access to water and 1·8% saline for several days previous to operation chose water in the immediate post-operative period of 6 hr (Table 2), though later on a considerable preference for saline developed.

Drinking appeared to be less urgent than the drinking which follows injection of hypertonic saline or a period of water deprivation, perhaps because of the increasing hypotonicity of the body fluids as drinking proceeds and because of the debilitating effect of acute hypotension. Nevertheless, the amounts of water drunk were quite large, often equal to half the total amount of water drunk in 24 hr by a normal rat allowed food and water *ad libitum*. The largest amount of water taken in the 6 hr immediately following caval ligation by an individual rat was about 30 ml. Unexpectedly, the peak of drinking occurred about 1 hr after the peak haemodynamic changes.

After infrarenal caval ligation there was almost as much drinking as after a ligature above the renal veins but renal function was much less affected. Urine flow increased as a result of the increased water intake and was greater than the control value. Nevertheless, there was some impairment of renal function as shown by the reduced electrolyte excretion, and this was especially evident in experiments (not reported here) in which an infrarenal caval ligation was combined with injection of hypertonic solutions of saline or sucrose.

The values of serum Na and osmotic pressure, the shortness of the interval between ligation and the commencement of drinking, and the fact that infrarenal caval ligation is an effective stimulus of drinking, do not support a theory that drinking is a consequence of oliguria. Nevertheless, the possibility was excluded by making animals anuric by submitting them to bilateral nephrectomy or to bilateral ureteric ligation (Table 3).

Neither procedure alone caused increased drinking, therefore drinking after caval ligation cannot be attributed to failure of excretion. However, it also became apparent that the kidneys are involved in drinking after caval ligation, because caval ligation in bilaterally nephrectomized rats was relatively ineffective as a stimulus of drinking, whereas caval ligation combined with bilateral ureteric ligation resulted in drinking.

TABLE 3. Drinking in the 6 hr which followed caval ligation when there had been interference with renal function by nephrectomy or by ureteric ligation

	Water drunk ml./100 g initial body wt.	Change in body wt. g/100 g initial body wt.
Caval ligation and bilateral nephrectomy (15)	1·60 ± 0·25	+ 0·54 ± 0·21
Sham caval ligation and bilateral nephrectomy (15)	1·20 ± 0·45	− 0·25 ± 0·20
Caval ligation and bilateral ureteric ligation (12)	3·04 ± 0·47	+ 1·76 ± 0·47
Sham caval ligation and bilateral ureteric ligation (15)	1·12 ± 0·30	+ 0·13 ± 0·55

Thus certain changes in the circulation without any initial alteration in over-all fluid and electrolyte balance can cause drinking, an effect best seen in animals with kidneys perfused by their own circulation, though these kidneys need not necessarily be secreting urine.

The long-term effects of caval ligation above the renal veins were followed in the eight rats of Table 2. These animals were kept in the individual metabolism cages where they had access to water and 1·8% saline. Standard rat diet was also provided except during the 6 hr period that immediately followed caval ligation.

Caval ligation had little effect on the long-term survival of the rats. By about the fifth post-operative day the animal's behaviour was indistinguishable from that of an unoperated rat, food and water intake were back to pre-operative values and the growth curve was returning to normal. Measurements from three rats, representative of the eight followed in this experiment, are given in Fig. 2.

After the initial burst of drinking, largely finished by about the third hour, there was little further drinking during the next 24–48 hr and intake of food also fell to very low values. Despite the aphagia and adipsia the

initial burst of drinking coupled with the persisting oliguria ensured a gain in weight by the animal in the first 24 hr after operation.

The increase in weight represented a net gain of fluid. This, together with the accumulated osmotic material resulting from the oliguria, eventually gave rise to a substantial diuresis as the circulation and renal function came back to normal with the opening up of collateral vessels.

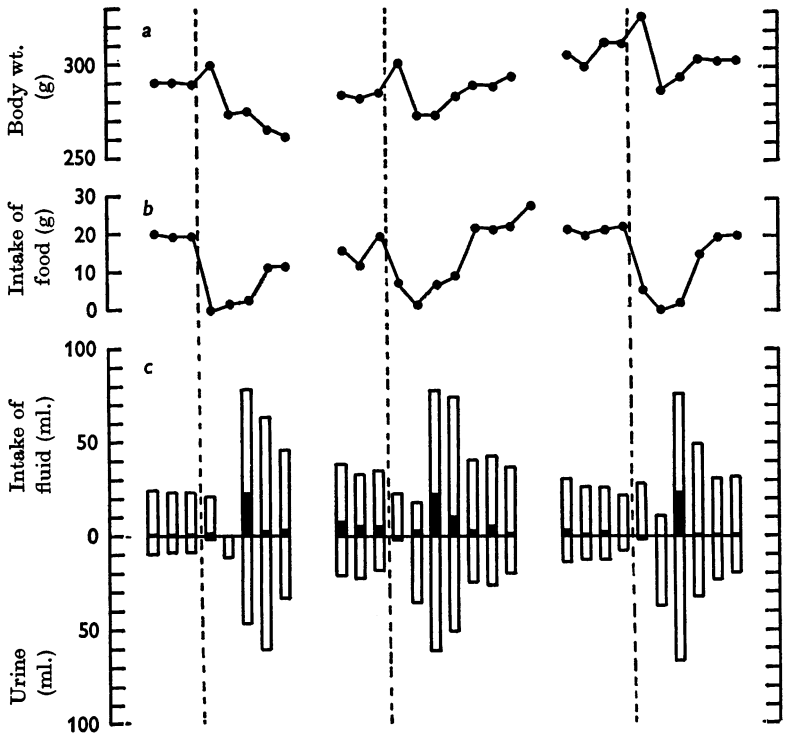

Fig. 2. (*a*) The body wt., (*b*) the food intake, (*c*) the total intake of fluid (open columns) and intake of 1·8 % saline (filled columns) plotted upwards, and output of urine plotted downwards, every 24 hr, of three rats subjected to caval ligation above the renal veins. The day of operation is indicated by the vertical interrupted line.

The serum osmotic pressure was elevated just before the onset of the diuresis but serum Na remained low, despite the abrupt fall in Na excretion in the oliguric phase, because Na intake also fell and because the animal was in positive water balance.

During the diuresis the body weight fell sharply and electrolyte was lost in the urine. A secondary polydipsia now developed in which the rat showed an increased preference for 1·8 % saline. This reached a peak usually on the third day after caval ligation and it always followed the

diuresis and loss of body weight. At about the same time the animal started to eat again though it was not until about the fifth day that intake of food had returned to normal. Delayed polydipsia and saline preference lasted 1–2 days followed by a gradual return to preligation values.

Constriction of the aorta or renal arteries as a stimulus to drinking. After complete occlusion of the inferior vena cava above the renal veins there was a generalized fall in mean arterial pressure, and there was also considerable venous engorgement in the kidneys so that damage easily resulted when they were mishandled. The damage caused in these experiments was probably slight because renal function returned to normal within a few days of ligation, as rapidly as the return of blood pressure to normal. There was also little risk of damage when the ligature was placed below the renal veins, since in this case there was no venous engorgement in the kidneys. Renal damage is therefore probably not a factor in this type of drinking, even though there is a potent dipsogen in the kidney (see later).

However, in view of the fact that drinking after caval ligation depended in large measure on the presence of the kidneys, it became necessary to determine to what extent the generalized circulatory changes contributed to drinking since it appeared that alterations in renal circulation alone might suffice.

A satisfactory, though technically fairly difficult way of reducing renal artery pressure is to constrict partially the abdominal aorta above the renal arteries. This procedure caused a fall in arterial pressure below the constriction and a slight rise in pressure above the constriction: there was no venous engorgement. In one group of controls the abdominal aorta was constricted in a similar manner below the renal arteries; in another group aortic constriction was carried out after bilateral nephrectomy. In both experimental and control groups the degree of aortic constriction is critical. If the ligature is too tight there is danger of widespread insufficiency of blood flow to the abdominal viscera and hind quarters. It was always obvious when this happened for the animal quickly became shocked and at autopsy evidence of widespread ischaemia was found. These animals were always killed immediately it became apparent that they were ill, and the results from them were discarded.

Aortic constriction above the renal arteries had an effect on water intake similar to that of caval ligation, with drinking starting about ½–1 hr after operation and continuing for several hours after this. Neither infrarenal aortic constriction nor aortic constriction after nephrectomy had any effect on water intake (Table 4). Urine flow fell after aortic constriction above the renal arteries and increased after infrarenal constriction as compared with sham operated controls (see Table 1); the

TABLE 4. Drinking and excretion in the 6 hr which followed partial constriction of the aorta above the renal arteries, constriction of the renal arteries, infrarenal aortic constriction, or aortic constriction after nephrectomy

	Water drunk ml./100 g initial body wt.	Change in body wt. g/100 g initial body wt.	Urine volume ml./100 g initial body wt.	Na μM/100 g initial body wt.	K μM/100 g initial body wt.
Aortic constriction above the renal arteries (25)	$4{\cdot}58 \pm 0{\cdot}31$	$+2{\cdot}60 \pm 0{\cdot}96$	$0{\cdot}53 \pm 0{\cdot}14$	$13{\cdot}5 \pm 4{\cdot}06$	$51 \pm 12{\cdot}9$
Constriction of the renal arteries (12)	$2{\cdot}18 \pm 0{\cdot}45$	$+0{\cdot}91 \pm 0{\cdot}44$	$0{\cdot}20 \pm 0{\cdot}05$	$29 \pm 8{\cdot}02$	$20 \pm 6{\cdot}67$
Aortic constriction below the renal arteries (10)	$0{\cdot}64 \pm 0{\cdot}21$	$-3{\cdot}19 \pm 0{\cdot}26$	$2{\cdot}24 \pm 0{\cdot}31$	$142 \pm 22{\cdot}73$	$180 \pm 21{\cdot}16$
Aortic constriction after nephrectomy (7)	$1{\cdot}04 \pm 0{\cdot}22$	$-0{\cdot}16 \pm 0{\cdot}18$	—	—	—

latter increase is possibly the result of an elevation in blood pressure in the vasculature above the constriction, including the renal arteries.

The amounts of water drunk by individual rats after aortic constriction above the renal arteries varied considerably. The mean intake of water in 6 hr of the five animals (Table 4) which drank the most was 12·18 ml./100 g body wt. and the actual amounts drunk by each of these animals were 16, 22·2, 26·7, 52·75 and 69 ml. Two animals showed signs of water intoxication towards the end of the experiment and were passing liquid faeces.

The correlation between the amount of water drunk and the arterial pressure difference across the constriction was poor, in part because the pressures measured at the end of the 6 hr experiment almost certainly did not reflect those prevailing at the time the animal was drinking. In seven experiments in which the mean amount of water drunk was 5·89 g/100 g body wt. the average mean pressures above and below the constriction were 118 and 76 mm Hg respectively.

The effect on drinking of aortic constriction above the renal arteries supports the hypothesis that alterations in the circulation to the kidneys may result in osmotically inappropriate drinking. The fall in arterial pressure was confined to below the constriction instead of involving the whole arterial bed as after caval ligation, and there was no venous congestion.

An attempt was made to restrict the circulatory changes still further by placing constrictions on both renal arteries. This procedure is the most direct way of producing circulatory changes confined to the kidneys. It is also the most difficult because the artery is small and in many animals oedema at the site of the constriction converted what was initially a partial blockage into a complete one. In view of these difficulties it was satisfactory to find that the procedure caused a significant increase in water intake though the effect was not as great as that which followed caval ligation or aortic constriction above the renal arteries (Table 4).

The effects of renal extracts on drinking. One interpretation of the effect on drinking of caval ligation, aortic constriction or constriction of the renal arteries is that these procedures cause the kidneys to release a humoral thirst factor into the circulation.

Simple saline extracts of renal cortex, renal medulla and liver from the rat were given by intraperitoneal injection to normal and to bilaterally nephrectomized rats. Extracts from the renal cortex caused increased drinking by nephrectomized rats but dipsogenic activity was not found in cortical extracts which had been boiled for 10 min, nor in extracts of renal medulla and liver (Table 5). Contrary to earlier reports, cortical extracts also caused animals with intact kidneys to drink, but the effect was much less than in nephrectomized rats (Table 5; Figs. 4 and 5).

TABLE 5. The effects of renal extracts on drinking. All experiments lasted 6 hr

	Equivalent wt. of fresh kidney injected g/100 g initial body wt.	Water drunk ml./100 g initial body wt.	Change in body wt. g/100 g initial body wt.	Urine volume ml./100 g initial body wt.
		(*a*) Normal rats		
Saline extract of rat kidney (6)	0·87	1·81 ± 0·76	−2·47 ± 0·50	2·56 ± 1·37
Isotonic saline (6)	—	0·33 ± 0·16	−4·21 ± 0·52	1·99 ± 0·21
		(*b*) Nephrectomized rats		
Saline extract of rat kidney (12)	0·58	6·31 ± 0·57	+5·33 ± 0·55	—
Purified extract of rat kidney (8)	0·67	4·62 ± 0·54	+3·53 ± 0·60	—
Purified extract of rat kidney and adrenalectomy (5)	0·53	3·85 ± 0·61	+2·69 ± 0·58	—
Saline extract of renin depleted rat kidney (10)	0·44	2·81 ± 0·45	+1·59 ± 0·43	—
Boiled extract of rat kidney (8)	0·48	0·76 ± 0·10	−0·40 ± 0·20	—

Cortical extracts had no effect on the intake of 1·8% saline when both water and saline were available to drink.

The renal dipsogen, if not identical with renin, resembles it so closely that it proved impossible to separate the two factors during the fractionation procedure used to make renin; pressor and dipsogenic activities were always present to the same degree in the particular fraction under consideration and absence of one type of activity meant absence of the other (Fig. 3).

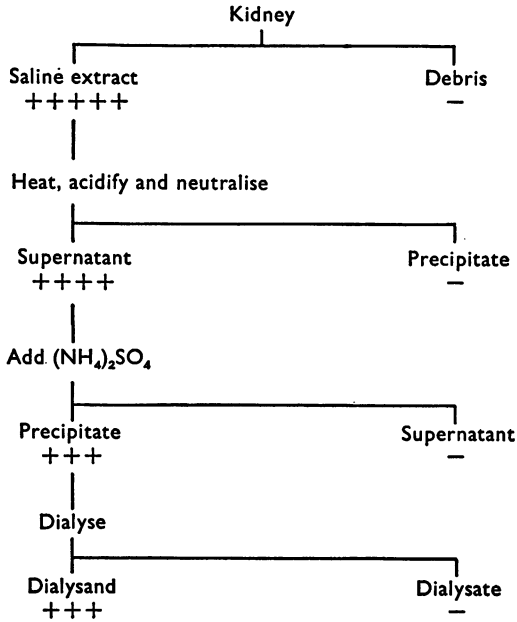

Fig. 3. Extraction procedure for the renal dipsogen. The presence or absence of dipsogenic and pressor activities is indicated by + or −.

When rats were injected subcutaneously with 10 mg DOCA daily for 10 days and were provided with 0·9% saline to drink, procedures which deplete the kidneys of renin, the amount of extractable drinking factor was also reduced (Table 5).

It seems therefore that the renal dipsogen and renin are the same substance. The recent finding (Fitzsimons & Simons, 1968) that angiotensin causes rats to drink, and that this action, like the pressor action of angiotensin is more pronounced after nephrectomy, supports this view.

The adrenal glands play no part in drinking induced by the renal dipsogen (Table 5) or by caval ligation (Table 1). On the one hand, bilateral adrenalectomy performed just before the drinking test had no effect on the response to either procedure. On the other hand aldosterone had no effect on the intake of water by normal and nephrectomized rats.

Experiments in which purified pig renin (Nutritional Biochemicals Corporation) was given by intravenous injection to normal and nephrectomized rats provide further support for the identity of renin and renal dipsogen. When renin was injected intravenously the animal started to drink almost immediately; there was very little latency and the time

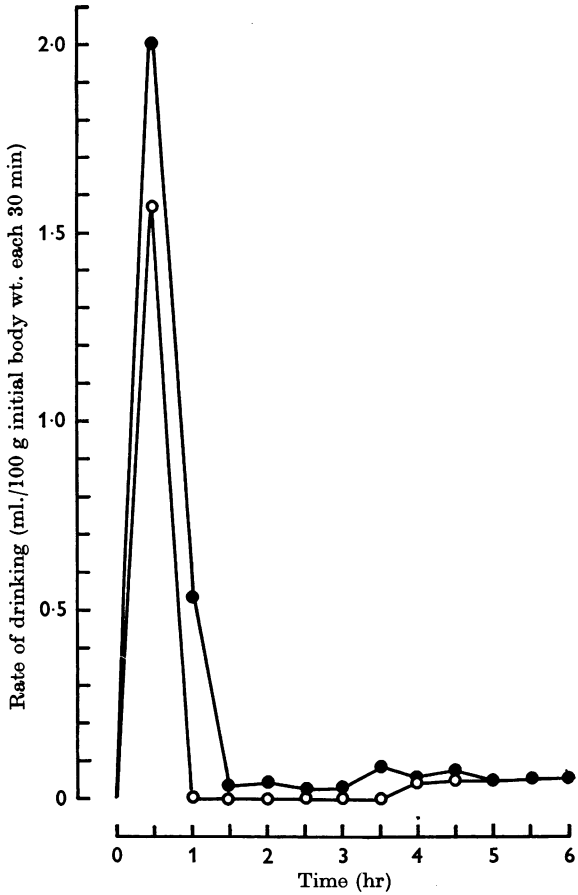

Fig. 4. Comparison of the rates of drinking in normal rats after the intravenous injection of (●) pig renin (ten results, 0·8–6·0 Goldblatt units) and of (○) hypertonic saline (four results, 3·9 % increase in osmotic pressure).

course of drinking was much the same as after intravenous hypertonic saline (Fig. 4). As was the case after intraperitoneal administration, more water was drunk by nephrectomized rats than by animals with intact kidneys (Fig. 5).

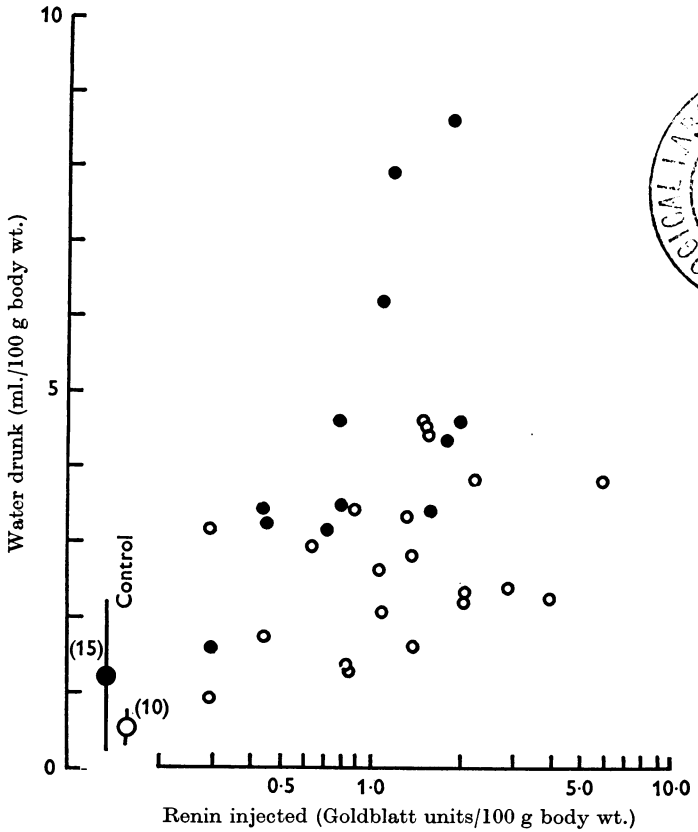

Fig. 5. The amounts of water drunk in 6 hr by normal (○) and by nephrectomized (●) rats after intravenous administration of different doses of pig renin. Each point is a single observation, except the controls which are mean values ± S.D. with the number of observations in parentheses.

DISCUSSION

The major part of the drinking which follows caval ligation, aortic constriction or constriction of the renal arteries seems to depend on alterations in the circulation to the kidneys. The increased intake of water is all the more impressive because it was usually accompanied by oliguria so that the animals ended the initial period of drinking in positive fluid balance and showed a fall in serum Na.

The marked diminution in urine flow, which occurred after most of these procedures, does not account for the drinking because, on the one hand neither bilateral nephrectomy nor bilateral ureteric ligation alone caused drinking, and on the other hand infrarenal caval ligation was an

effective stimulus of drinking even though renal excretory function was only slightly affected.

The kidneys were responsible for most if not all of the drinking which followed constriction of the renal arteries. They also contributed largely to the extra drinking which followed aortic constriction above the renal arteries, or caval ligation, because nephrectomized rats subjected to the same procedures showed little or no extra drinking. In fact only after caval ligation, when the circulatory changes were the most widespread involving the whole vasculature, did nephrectomized rats drink a little more than control nephrectomized animals; even so, the effect of caval ligation was much less than in intact animals. Conversely, in animals made anuric by ureteric ligation, caval ligation caused extra drinking.

These experiments show that alteration in the circulation to the kidneys unaccompanied by any change in the over-all fluid and electrolyte balance of the animal is a sufficient stimulus to drinking in the rat. The kidney need not be forming urine but it must be perfused by the animal's own circulation. The presence in the kidney of a potent dipsogen makes it attractive to suppose that certain stimuli of drinking exert part of their effect through release of this dipsogen into the circulation.

The suggestion that the kidney may be concerned in thirst was first made by Linazasoro, Jiménez Díaz & Castro Mendoza (1954). These authors found that nephrectomized rats provided with water but not food lost weight, but that the loss of weight could be prevented by giving the animals glycerine extracts of pig kidney. The loss of weight was less when the animals were made anuric by ureteric ligation. The authors considered that the nephrectomized rats lost weight because the sensation of thirst was impaired owing to the absence of a renal thirst factor. The nature of this humoral substance was not stated, but in a later study it was said not to be renin (Jiménez Díaz, Linazasoro & Merchante, 1959).

The experiments of Nairn, Masson & Corcoran (1956), and of Asscher & Anson (1963), support this suggestion of a renal thirst factor, since both groups found that renal extracts caused increased intake of water by nephrectomized animals. However, these authors were primarily concerned with the pathological effects of renal extracts in causing serous effusions into the various cavities of the body and in causing damage to blood vessels. Nairn and his colleagues considered that the active principle which produced serous effusions was renin since they found that very large doses of renin (6·5 Goldblatt units/8 hourly for 5–6 doses) administered subcutaneously caused the same syndrome. According to them this action of renin was not to be attributed to its pressor action because fairly pure angiotensin and noradrenaline did not cause effusions. Asscher & Anson (1963) did not identify the active principle in their extracts but showed

that it was confined to the cortex of the kidney and that it was destroyed by boiling. Later, however, Cuthbert, Asscher & Henry Jones (1966) showed that the renal vascular permeability factor is probably renin.

In the present experiments it proved impossible to separate the pressor and dipsogenic activities contained in pig kidney and rat kidney, using the rat as the test animal and following the extraction procedure of Haas *et al.* (1954). Both activities were found only in the renal cortex, both were destroyed by boiling and both activities were more pronounced in the nephrectomized rat. Commercially available pig renin was fully effective in causing rats to drink and, as with renal extracts, this action was greater after nephrectomy.

The increased pressor activity of renal extracts in nephrectomized rats may be attributable in part to increased angiotensin formation resulting from the higher level of plasma renin substrate prevailing after nephrectomy. Carretero & Gross (1967) found that there was a significant increase in concentration of substrate 2 hr after nephrectomy and that maximum values (more than four times the initial value) were attained within 8 hr of operation. The increased dipsogenic activity of renal extracts after nephrectomy may have a similar explanation in view of the recent evidence that angiotensin stimulates drinking (Fitzsimons, 1966; Fitzsimons & Simons, 1968). However, other as yet unknown factors, including inactivation of the extract by the kidney, cannot at present be excluded.

It seems highly likely therefore that the renal dipsogen and renin are identical, and though it remains uncertain to what extent this substance participates in the control of water intake under normal conditions, it is worth noting that most extracellular stimuli of thirst are also known to augment renin secretion.

The smaller doses of renin used here could lie within the normal endogenous range. Schaechtelin, Regoli & Gross (1964) found that the renin content of plasma in normal rats is 0·15 Goldblatt units/100 g of rat and in hypertensive rats is about 0·6 units/100 g of rat. The fact that these doses cause a detectable increase in water intake by animals in normal water balance makes it reasonable to suppose that renin is a significant factor in extracellular thirst, though since hypovolaemic stimuli cause drinking in nephrectomized rats, it is clearly not the only one.

There are at least three possible ways in which the renin angiotensin system could play a role in drinking induced by extracellular stimuli (Fig. 6).

In the first place there is the possibility that the setting in action of a vasoactive system as powerful as the renin angiotensin system might increase the sensitivity of the vascular stretch receptors to the existing hypovolaemia so that the sensory discharge from these receptors is aug-

mented. According to this hypothesis the circulatory changes which follow caval ligation, aortic constriction or hypovolaemia, upset the balance of nervous information from a variety of receptors the function of which is to register extracellular volume. In the absence of the kidneys this sensory imbalance is small and leads to a small and insufficient intake of fluid. But if the kidneys are present enough angiotensin is generated to enhance

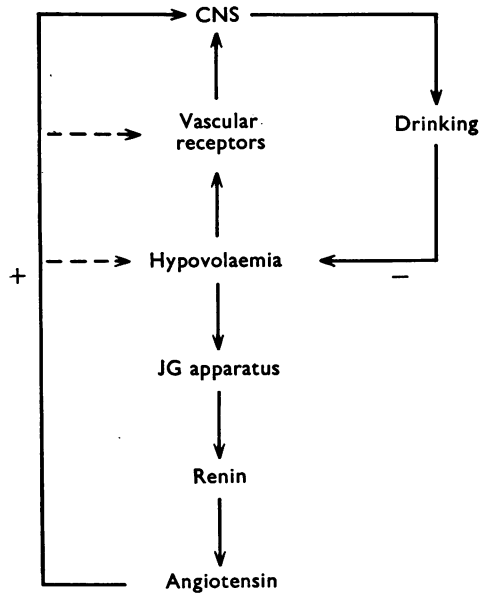

Fig. 6. Possible ways in which the renin angiotensin system may play a role in drinking caused by extracellular stimuli.

the sensory response so that drinking now ensures that the full and appropriate amount of water is taken.

A second way in which renin could act is through its effect on plasma volume. In large doses renin causes increased capillary permeability and a fall in plasma volume. According to Peart (1965) the factor affecting permeability is renin itself though it can be shown that angiotensin also has this effect. No matter which substance is responsible for the increased permeability, the effect on plasma volume would be to accelerate drinking by exaggerating the hypovolaemia which caused secretion of renin and drinking in the first place.

It is doubtful, however, whether there is enough endogenous renin secretion to affect plasma volume to any significant extent. Furthermore, it seems inherently improbable that a mechanism of this kind would operate within the physiological range of renin secretion.

A third possibility is that renin and/or angiotensin have some central

action. Booth (1968) has reported that angiotensin (0·675 μl. of a 2·5 μg/μl. solution) placed directly in the rostral part of the lateral hypothalamus sometimes caused rats to drink substantial amounts of water, and this has been recently confirmed (Epstein, Fitzsimons & Simons, 1969), using much smaller (down to 10 ng) doses of angiotensin. It is of course very difficult to interpret the results of direct chemical stimulation of the hypothalamus because, though the quantity of active agent involved is usually small, the local concentration is exceedingly high. Nevertheless, even though lower and more physiological concentrations of angiotensin may have no direct stimulating effect on the hypothalamus, the possibility remains that angiotensin may lower the threshold of the nervous centres concerned in drinking sufficiently to ensure that enough water is drunk in response to thirst stimuli arising elsewhere in the body.

Yet another possibility in view of the involvement of the renin angiotensin system in the control of aldosterone (though perhaps not in the rat) is that the response is mediated through secretion of adrenal cortical hormones. However, this appears to be ruled out by the fact that adrenalectomy did not prevent drinking after caval ligation or injection of renal extract, and by the fact that aldosterone had no effect on water intake. It is possible however that aldosterone may be concerned in the delayed Na appetite which follows caval ligation since in extremely large doses it has been shown to increase consumption of isotonic saline (Wolf & Handal, 1966).

A noteworthy feature of the immediate drinking caused by caval ligation and administration of renin is that, when water and saline were both offered to drink, the solution chosen by the rat was always water, despite an increasing hypotonicity as drinking proceeded. Only during the delayed polydipsia was there an increased preference for saline, probably attributable to an increasing Na deficit caused by a fall in dietary Na intake and an accelerated loss of Na as urine flow recovered. There is a similar delay in the onset of saline preference after hyperoncotic peritoneal dialysis; during and immediately after dialysis the solution of choice is water, only much later does a marked preference for saline develop.

The early lack of interest in a solution which on the face of it appears more suited to the animals' needs may be related to the fact that the mechanism controlling Na intake is non-regulatory. The amount of salt ingested depends not only on the Na deficit, but also on the concentration of the saline offered (Weiner & Stellar, 1951). Precise regulation of the Na content of the body appears to be renal, and preference for saline provides a means whereby Na is introduced into the animal. After a hypovolaemic stimulus, however, there is an immediate need for restoration of volume, primarily in order to maintain the circulation. Water is

best suited for this because it is a substance of uniform composition which presents no problem of variable palatability and because the mechanisms controlling its intake are quantitative.

REFERENCES

ASSCHER, A. W. & ANSON, S. G. (1963). A vascular permeability factor of renal origin. *Nature, Lond.* **198**, 1097–1099.

BOOTH, D. A. (1968). Mechanism of action of norepinephrine in eliciting an eating response on injection into the rat hypothalamus. *J. Pharmac. exp. Ther.* **160**, 336–348.

CARRETERO, O. & GROSS, F. (1967). Renin substrate in plasma under various experimental conditions in the rat. *Am. J. Physiol.* **213**, 695–700.

CUTHBERT, M. F., ASSCHER, A. W. & HENRY JONES, J. (1966). Characterization of a vascular permeability factor of renal origin. *Clin. Sci.* **31**, 325–336.

EPSTEIN, A. N., FITZSIMONS, J. T. & SIMONS, BARBARA J. (1969). Drinking caused by the intracranial injection of angiotensin into the rat. *J. Physiol.* **200**, 98–100*P*.

FALK, J. L. (1961). The behavioral regulation of water-electrolyte balance. In *Nebraska Symposium on Motivation*, pp. 1–33. Lincoln: University of Nebraska Press.

FITZSIMONS, J. T. (1961). Drinking by rats depleted of body fluid without increase in osmotic pressure. *J. Physiol.* **159**, 297–309.

FITZSIMONS, J. T. (1964). Drinking caused by constriction of the inferior vena cava in the rat. *Nature, Lond.* **204**, 479–480.

FITZSIMONS, J. T. (1966). Hypovolaemic drinking and renin. *J. Physiol.* **186**, 130–131*P*.

FITZSIMONS, J. T. (1967). The kidney as a thirst receptor. *J. Physiol.* **191**, 128–129*P*.

FITZSIMONS, J. T. & SIMONS, BARBARA J. (1968). The effect of angiotensin on drinking in the rat. *J. Physiol.* **196**, 39–41*P*.

HAAS, E., LAMFROM, H. & GOLDBLATT, H. (1954). A simple method for the extraction and partial purification of renin. *Archs Biochem. Biophys.* **48**, 256–260.

JIMÉNEZ DÍAZ, C., LINAZASORO, J. M. & MERCHANTE, A. (1959). Further study of the part played by the kidneys in the regulation of thirst. *Bull. Inst. med. Res.* (*Madr.*) **12**, 60–67.

LINAZASORO, J. M., JIMÉNEZ DÍAZ, C. & CASTRO MENDOZA, H. (1954). The kidney and thirst regulation. *Bull. Inst. med. Res.* (*Madr.*) **7**, 53–61.

NAIRN, R. C., MASSON, G. M. C. & CORCORAN, A. C. (1956). The production of serous effusions in nephrectomized animals by the administration of renal extracts and renin. *J. Path. Bact.* **71**, 155–163.

OATLEY, K. (1964). Changes of blood volume and osmotic pressure in the production of thirst. *Nature, Lond.* **202**, 1341–1342.

PEART, W. S. (1965). The renin-angiotensin system. *Pharmac. Rev.* **17**, 143–182.

SCHAECHTELIN, G., REGOLI, D. & GROSS, F. (1964). Quantitative assay and disappearance rate of circulating renin. *Am. J. Physiol.* **206**, 1361–1364.

SEMPLE, R. E. (1952). Compensatory changes in the rat following removal of electrolytes, by intraperitoneal dialysis. *Am. J. Physiol.* **168**, 55–65.

STRICKER, E. M. (1966). Extracellular fluid volume and thirst. *Am. J. Physiol.* **211**, 232–238.

SWANSON, P. P., TIMSON, G. H. & FRAZIER, E. (1935). Some observations on the physiological adjustment of the albino rat to a diet poor in salt when edestin is the source of dietary protein. *J. biol. Chem.* **109**, 729–737.

WEINER, I. H. & STELLAR, E. (1951). Salt preference of the rat determined by a single stimulus method. *J. comp. physiol. Psychol.* **47**, 7–13.

WOLF, G. & HANDAL, P. J. (1966). Aldosterone-induced sodium appetite: dose–response and specificity. *Endocrinology* **78**, 1120–1124.