J. Physiol. (1979), **292**, *pp.* 421–428
With 2 text-figures
Printed in Great Britain

PLASMA ACTIVE AND INACTIVE RENIN IN THE RABBIT: EFFECT OF DIETARY SODIUM DEPLETION AND REPLETION

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(*Received 28 September 1978*)

SUMMARY

1. Active and acid-activatable (inactive) renins were measured in rabbit plasma under control conditions and during sodium depletion with subsequent repletion.

2. Active renin increased by 97 % during sodium depletion and returned to control levels on repletion. Both changes were complete within 1 day of changing the diet.

3. During dietary sodium depletion inactive renin levels initially fell to zero and then increased until, after 13 days, inactive renin was again 10 % of total renin levels, a proportion comparable to the control values.

4. Sodium repletion caused plasma inactive renin to return to control levels over about 13 days, a quite different time course to active renin. Therefore, in the first phase of repletion the proportion of total renin in the inactive form rose to 19 %.

5. These changes are discussed in relation to concurrent changes in sodium, potassium and water metabolism.

INTRODUCTION

The existence of an inactive, but activatable, form of renin was first demonstrated by Lumbers (1971) during studies on renin in human amniotic fluid. Subsequently, it has been shown that two forms of renin exist in the plasma of several species including man (Day & Luetscher, 1975; Skinner, Cran, Gibson, Taylor, Walters & Catt, 1975; Leckie, McConnell, Grant, Morton, Tree & Brown, 1977; Sealey, Moon, Laragh & Atlas, 1977*a*; Derkx, Wenting, Man in't Veld, Verhoeven & Schalekamp, 1978), the dog (James & Hall, 1974) and the rabbit (Richards, Grace, Noble & Munday, 1979). Similar forms of renin have been isolated from extracts of kidney and several other tissues. A working hypothesis is that high molecular weight (inactive) and low molecular weight (active) forms of renin are synthesised in the kidney and both forms are secreted from there into the blood where activation of inactive renin may occur. Almost every aspect of this working hypothesis is still contentious.

Experimentally, inactive forms of renin can be activated in one of three ways. The most widely used is activation by acidification (Day & Luetscher, 1975; Skinner *et al.*

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0022-3751/79/4420-0759 $01.50 © 1979 The Physiological Society

1975; Leckie *et al.* 1977; Richards *et al.* 1979), but cryoactivation (Osmond, Ross & Scaiff, 1973; Sealey *et al.* 1977*a*) and activation with trypsin (Morris & Lumbers, 1972; Cooper, Murray & Osmond, 1977) have been reported. Under physiological conditions it is now suggested that activation of renin is brought about by a neutral serine protease enzyme which is normally bound to an inhibitor. The experimental methods of activation described above are considered to destroy the inhibitor (Atlas, Sealey & Laragh, 1978).

Interconversion of active and inactive renins, or their differential secretion from the kidney, therefore presents a novel site for control of the renin–angiotensin system. Little is currently known of the response of circulating levels of the two forms of renin to altered physiological circumstances. James & Hall (1974), in a study using dogs, showed that in response to haemorrhage there was a parallel increase in circulating levels of both forms of renin and we have reported similar findings in rabbits (Richards *et al.* 1979). James & Hall (1974) also found that acute sodium depletion, using the diuretic frusemide, caused inactive renin levels to fall to zero. A decrease in the proportion of total renin which was in the inactive form has recently been reported in human subjects fed a low sodium diet (Hsueh, Carlson, Luetscher & Grislis, 1978; Millar, Leckie, Semple, Morton, Sonkodi & Robertson, 1978). In another study of the responses of human plasma renin levels to acute stimuli such as isoprenaline infusion, tilting or vasodilation stimulated by diazoxide, it was found that there was a relatively much larger increase in active than inactive renin. Under steady-state circumstances, however, there was a close correlation between levels of the two forms of renin (Derkx, Wenting, Man in't Veld, Van Gool, Verhoeven & Schalekamp, 1976).

We have investigated dietary sodium depletion in rabbits with a view to correlating the progressive changes in sodium status with the levels of active and inactive renin in plasma. The time course of the experiment covered both short term changes over the first few days of sodium depletion and the establishment of a new stable situation. The details of the design of the experiments, and the alterations which occurred in sodium and potassium handling by the kidney and gut are described in the preceding paper in this journal (Grace, Munday & Noble, 1979).

METHODS

Five male and four female New Zealand White rabbits in the weight range 3·0–4·5 kg were maintained in metabolism cages throughout the experiment. The animals were weaned onto a synthetic diet in which a sodium concentration of 140 m-mole/kg was achieved by adding sodium chloride, before pelleting, to a low sodium diet containing 6 m-mole/kg. Potassium content of both diets was 260 m-mole/kg. When the animals reached maturity, measurements were made, over a control period of 18 days, of diet and water intake together with urinary and faecal excretion of sodium and potassium. Blood pressure was monitored using a Grant–Rothschild capsule placed on the central ear artery. After the first control period the animals were sodium depleted by feeding the low sodium diet for an 18-day period and then sodium-repleted by restoring the animals to the control diet for a further eighteen days. Care was taken to ensure a constant potassium intake throughout the course of the experiment. Further details of the experimental protocol are given in the preceding paper (Grace *et al.* 1979).

Blood samples (6 ml.) were retained every 3 days for the estimation of total and active plasma renin levels. Inactive renin represented the difference between these two sets of measurements.

For total renin estimation, the plasma sample was dialysed to pH 3·3 against a glycine-HCl buffer (glycine 50 mM; HCl 7 mM; EDTA Na_2 5 mM) for 24 hr at 4 °C. The dialysis sacs were then transferred to the same buffer at 32 °C for 60 min. This was followed by dialysis back to pH 7·4 against a phosphate buffer (NaH_2PO_4 14 mM; Na_2HPO_4 83 mM; EDTA Na_2 5 mM) for 24 hr at 4 °C. For measurements of active renin, the acidification and heating steps were omitted and the plasma sample was dialysed at pH 7·4 for 48 hr. Recovery of pre-activated exogenous renin for both dialysis protocols was 91 %. After dialysis both sets of samples were processed identically for the measurement of renin activity. Aliquots (0·2 ml.) of each sample were incubated at 37 °C with excess sheep renin substrate (Skinner, 1967) in the presence of disodium EDTA (2·7 mM) and Trasylol (FBA) (200 u./ml.). Angiotensin generation rate over a time course up to 24 hr was estimated using a bracketing dose bioassay against angiotensin II amide (Hypertensin-Ciba) using a nephrectomized, ganglion-blocked rat blood-pressure preparation (modified after Peart, 1955). Angiotensin generation showed a linear relationship with time. Eighteen replicate plasma renin samples had a mean variation of 10·0 ± 2·6 %. One renin unit was taken to be a generation rate of 1 ng angiotensin II equivalent per ml. plasma sample per hour.

Statistical analysis of results was by paired Student's *t* test.

RESULTS

Results for estimations of active renin levels are shown in Fig. 1*A*. During dietary sodium depletion there was a 97 % rise in plasma renin activity ($P < 0·001$). On return to the control diet, active renin levels returned to normal. The blood samples taken 24 hr after each of these changes in diet showed that a maximum change in plasma active renin had already occurred. An increase in plasma renin levels in response to dietary sodium depletion has been reported by many other groups and is associated with both an increase in kidney renin content and raised sensitivity of the renin release mechanisms. (Bunag, Page & McCubbin, 1966; Fray, 1978).

Total renin levels are shown in Fig. 1*B*. From this data, after subtraction of the active renin (Fig. 1*A*) the levels of inactive renin were derived (Fig. 1*C*). Plasma inactive renin did not alter significantly from a mean of 0·3 ± 0·04 renin units during the initial control period. After changing to the low sodium diet the inactive renin levels fell to zero. During the later part of the low sodium diet period, inactive renin levels rose to a maximum of 0·8 ± 0·3 renin units so that there was a significant difference ($P < 0·01$) between sample 7 (day 19) and sample 12 (day 34). It appeared that after about thirteen days on the low sodium diet a new stable level of inactive renin had been achieved. At this time the urinary and faecal excretion of sodium and potassium, urine volume and water intake were also constant at a new level. On sodium repletion, achieved by refeeding the control diet, inactive renin levels initially remained high and only slowly returned to base line over a period of about 13 days. Control levels were eventually re-established and sample 18 (day 52) was significantly lower than sample 13 (day 37) ($P < 0·05$). The time course for the changes in inactive renin contrasts markedly with the corresponding active renin levels shown in Fig. 1*A*. In this case a new stable active renin level was achieved within 1 day of the dietary change.

The values for absolute levels of inactive renin shown in Fig. 1*C* are of course obtained against a background of changing total renin levels. We have therefore also expressed the inactive renin levels as a percentage of total renin (Fig. 2). Under control conditions inactive renin was about 10 % of total plasma renin and this fell to

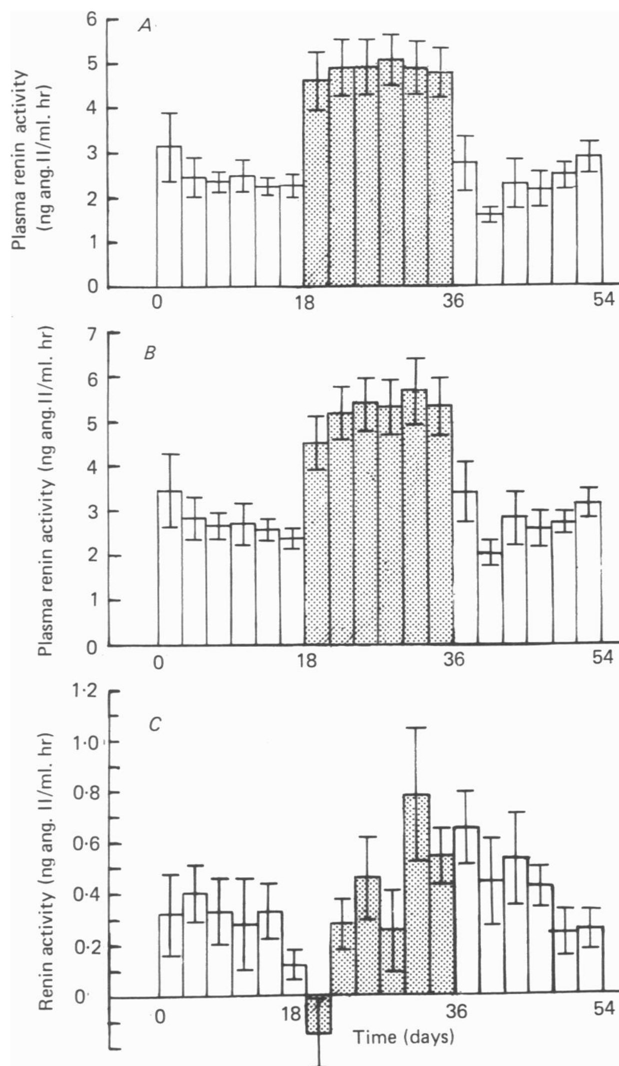

Fig. 1. *A*, plasma active renin in rabbits fed control diet (days 0–18), during sodium depletion (stippled area, days 19–36) and during sodium repletion (days 37–54). The increase in active renin was achieved 1 day after feeding the depletion diet. *B*, total plasma renin levels. This was measured after dialysis of plasma samples to pH 3·3 and represents the sum of active and inactive renins. *C*, plasma inactive renin levels. This data was derived by subtracting active renin levels (top histogram) from total renin levels (middle histogram). Attention is drawn to the difference in time course between these changes and those for active renin (top histogram).

zero when the animals were changed to the low sodium diet ($P < 0{\cdot}05$). The proportion of renin in the inactive form then rose to 19 % ($P < 0{\cdot}02$) at the beginning of sodium repletion and during the later part of this period the proportion of inactive renin again fell to control values.

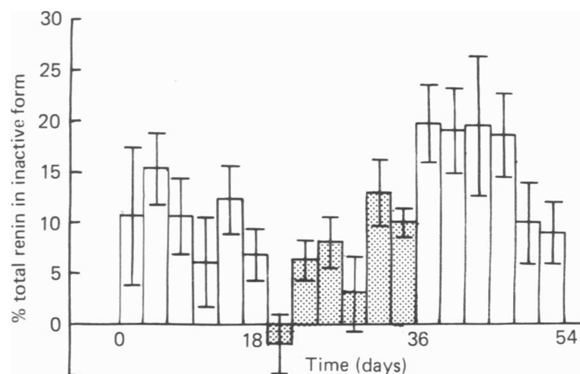

Fig. 2. Plasma inactive renin expressed as a percentage of total renin. Rabbits were fed control diet (days 0–18), a low sodium diet (stippled area, days 19–36) and then sodium repleted with the control diet (days 37–54).

DISCUSSION

The identification of inactive forms of renin in plasma and kidney extracts raises the possibility that interconversion, or differential secretion, of active and inactive renin may be a new adaptive level for control of the renin–angiotensin system. The results presented in this paper support this idea.

Increased levels of plasma active renin following dietary sodium depletion have been widely studied and an increase of 97 % (Fig. 1*A*), which returned to control values on sodium repletion, was found in the present study. New stable levels were reached by the time the first blood sample was taken 24 hr after each dietary change. Inactive renin levels followed a different pattern (Fig. 1*C*). The initial response to sodium depletion was for inactive renin to disappear. Then, over a period of 13 days, inactive renin increased until it again formed the same proportion of total plasma renin as in the control period. In the intermediate phase the proportion of renin in the inactive form was reduced because active renin had already increased (Fig. 2). Care must therefore be taken to consider the time course when interpreting the effects of sodium depletion, or any other stimulus, on the levels of inactive renin in plasma. This may partly explain why no clear pattern has emerged from the very few studies of interactions between sodium depletion and inactive renin levels so far reported. Hsueh *et al.* (1978), in a study of dietary sodium depletion in normal human subjects, found that, 5 days after the start of depletion, both forms of renin had increased but the proportion of total renin in the inactive form had fallen from 82 to 62 %. This is similar in pattern and time course to our data from rabbits. It is possible that if dietary sodium restriction for the human subjects had been continued for a further period, a new stable situation would have been reached with both active and inactive renin increased to the same degree.

The results presented in this paper are those for active and inactive renin measurements but concurrent estimations of electrolyte and water balance are described in the preceding paper (Grace, Munday & Noble, 1979). The initial response to sodium depletion, a fall in inactive renin levels to zero, can be correlated with other changes

discussed in this paper. There was a large negative sodium balance and a positive potassium balance at the time inactive renin disappeared from the circulation. In addition, both urine volume and water consumption had decreased. In view of the time course for this group of changes it is tempting, as others have done, to postulate a receptor within the splanchnic bed controlling these changes (Sealey *et al.* 1977*a*). Central to this hypothesis was the proposal that human inactive renin (prorenin) is largely extrarenal in origin, a suggestion based mainly on the observation of substantial amounts of prorenin in the plasma of anephric human subjects (Sealey, White, Laragh & Rubin, 1977*b*). We have been unable to confirm this suggestion in rabbits however as, one day after bilateral nephrectomy, no inactive renin could be detected in plasma (Richards *et al.* 1979).

The slow increase in plasma inactive renin levels in the later phase of sodium depletion most closely parallels the concurrent changes in urine volume and water intake. The alterations in sodium handling by the kidney occurred much more quickly than the changes in inactive renin. Dramatic alterations in potassium excretion were not seen in these experiments as dietary intake of potassium was constant on both diets.

In order to establish the mechanisms involved in the control of plasma inactive renin levels it will be necessary to determine whether alterations in circulating levels reflect changed secretion, and ultimately changed synthesis, of the two types of renin stored in either the kidney or possible extrarenal sources. The alternative explanation, is that activation of inactive renin occurs in the plasma and this is the variable control site determining the balance between circulating active and inactive renin. A clue to possible mechanisms, which supports the notion of changes in synthesis within the kidney, comes from the work of de Senarclens, Pricam, Banichahi & Vallotton (1977). This group studied inactive renin in rat kidneys. Release of renin was stimulated by bilateral adrenalectomy and dietary sodium depletion. Renin release was then abruptly blocked by 48 hr substitution treatment with DOCA and dietary sodium loading. An increase was found in the amount of inactive renin in the kidneys. It was suggested that the newly synthesized renin, produced in response to sodium depletion, is stored bound to a protein. This renders the renin inactive when the rats are sodium loaded. The experimental model used by de Senarclens *et al.* can be compared to the switch from low sodium to control diet in our own study. A rise in the proportion of plasma renin which was inactive was found. Plasma levels may therefore reflect the kidney levels of the two types of renin.

Under control conditions the acid-activatable form of renin in rabbit plasma represents 10–15 % of total plasma renin. This value compares closely with the level of 15 % in the rat reported by Vandongen, Poessee, Strang & Birkenhager (1977). It was suggested by these authors that anaesthesia, which is known to raise circulating renin levels, may cause a proportionally greater rise in active renin compared to the inactive form. However, comparison of the data we report here for samples taken from conscious rabbits with our previous study using urethane-anaesthetized rabbits (Richards *et al.* 1979) shows the same proportion of inactive renin in both cases. Absolute levels of both forms or renin are higher in anaesthetized animals.

It appears then that circulating levels of active and inactive renin may alter independently. Changes in inactive renin can occur rapidly but may take much

longer than active renin to reach a new stable level during adaptation to changed physiological circumstances. In the case of dietary sodium depletion, when such a stable situation is reached the relative proportions of the two forms are comparable to normal.

This investigation was carried out while S.A.G. was in receipt of a Medical Research Council Studentship. We are also grateful to the National Kidney Research Fund for financial support.

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