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SECRETION CONTROL FOR ACTIVE AND INACTIVE RENIN: EFFECTS OF CALCIUM AND POTASSIUM ON RABBIT KIDNEY CORTEX SLICES

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SUMMARY

1. Release of active and inactive renin by rabbit kidney cortex slices was investigated. Inactive renin was estimated as the increase in renin activity after acidification (pH 2·8) of slice supernatant solutions.

2. In Ca^{2+}-free media, release of both active and inactive renin was increased but the changes in inactive renin were more marked. The percentage of total renin released which was inactive ranged from 8·3% ($[Ca^{2+}] = 9·2$ mM) to 34·5% (zero $[Ca^{2+}]$) with a linear relationship ($r = -0·96$) over the range of $[Ca^{2+}]$ studied.

3. Depolarizing media ($[K^+] = 20$ mM) suppressed release of inactive renin more than release of the active form. This effect, for both forms of renin, was lost when Ca^{2+} ions were omitted from the incubation media. This suggests that influx of Ca^{2+} ions was responsible for the reduced renin secretion following depolarization of the juxtaglomerular cells.

4. Reducing the $[K^+]$ of the incubation buffer from 5·7 mM (control) to 1·0 mM did not alter active renin but increased release of inactive renin. Low $[K^+]$ media abolished the stimulatory effect of low $[Ca^{2+}]$ on release of both forms of renin.

5. In incubation media with low $[Ca^{2+}]$ or with low $[K^+]$ the mixture of renins released by the kidney cortex slices correlated with that found in extracts of non-incubated kidney: that is, about 35% of the total renin was in the inactive form.

6. Mechanisms controlling the secretion of active and inactive renins by the kidney are at least partially independent. Differential secretion of the two forms, perhaps linked to regulation of the activation of inactive renin before release, appears to be the basis of a control step in the over-all expression of the renin–angiotensin system. This may be coupled in some way to juxtaglomerular cell Na^+ ion flux.

INTRODUCTION

Secretion of renin by the juxtaglomerular cells of the kidney is thought to be partly regulated via voltage-sensitive Ca^{2+} channels in the cell membrane. Influx of Ca^{2+} ions inhibits renin secretion. This concept is based on studies using a variety of *in vitro* kidney preparations, including cortex slices (Park & Malvin, 1978; Churchill, 1981; Park, Han & Fray, 1981), isolated perfused kidneys (Van Dongen & Peart, 1974; Fray, 1977; Ettienne & Fray, 1979) and isolated glomeruli (Baumbach & Skøtt,

1981). Exposure to high [K^+] (square brackets denote concentration throughout) inhibits renin secretion by such preparations, probably as a result of depolarization of the juxtaglomerular cells (Fishman, 1976) and consequent influx of Ca^{2+} ions (Fray, 1978; Park & Malvin, 1978; Churchill, 1980). The inhibitory effects on renin release of angiotensin II (Van Dongen & Peart, 1974; Churchill, 1980), ouabain (Churchill, 1979) and antidiuretic hormone (Van Dongen & Peart, 1974) all appear to be dependent on influx of Ca^{2+} ions. Studies using Ca^{2+} ionophores (Baumbach & Leyssac, 1977; Fynn, Onomakpome & Peart, 1977) and Ca^{2+} antagonist drugs such as verapamil (Park *et al.* 1981), D600 (Churchill, 1980) and diltiazem (Churchill, McDonald & Churchill, 1981) have all provided support for the concept of an inhibitory role for Ca^{2+} influx into juxtaglomerular cells in the control of renin release. Renin secretion mediated by β-adrenoceptors is not affected by Ca^{2+} antagonists and cyclic AMP may be the secondary messenger in this case, with a possible subsequent role for Ca^{2+} ions (Churchill & Churchill, 1982).

The studies described above have all been concerned with secretion of the enzymatically active form of renin. The kidney of many mammalian species also secretes an inactive, but activatable renin. Work on the structure and interconversion of active and inactive renin has been reviewed (Sealey, Atlas & Laragh, 1980; Leckie, 1981) but a simple consensus viewpoint has yet to emerge. Differential secretion of the two forms of renin from the kidney may provide the basis for a physiologically important control step in the renin system.

We have previously shown that isoprenaline stimulates the release of active renin by rabbit kidney cortex slices, whilst the secretion of inactive renin remains unchanged (Richards, Noble & Munday, 1981*b*). Reducing [Na^+] in the incubation media stimulated active renin secretion but inhibited release of the inactive form (Munday, Noble & Richards, 1982). We have therefore investigated the secretion by kidney cortex slices of both active and inactive renin in response to changes in the [Ca^{2+}] of incubation media. In addition, the effects of increasing and decreasing [K^+] were studied in the presence and absence of Ca^{2+} ions. In all cases, Ca^{2+} depletion of slices was studied both in media in which Ca^{2+} was omitted and in buffers to which EGTA had been added with the aim of depleting Ca^{2+} in the extracellular space. Acidification to pH 2·8 was used to activate inactive renin.

A preliminary report of this work has already been published (Ginesi, Munday & Noble, 1981).

METHODS

The preparation of rabbit kidney cortex slices and evaluation of their viability have been fully described elsewhere (Munday *et al.* 1982). Briefly, slices were incubated for 90 min at 37 °C in Krebs–Ringer bicarbonate buffer (pH 7·4). After incubation, active and inactive renins present in slice supernatants and slice extracts were determined.

The ionic composition of the unmodified Krebs buffer was as follows: [Na^+], 133 mM; [K^+], 5·7 mM; [Cl^-], 117 mM; [Ca^{2+}], 2·3 mM; [HCO_3^-], 23 mM; [$H_2PO_4^-$], 1·1 mM; [SO_4^{2-}], 1·1 mM; [Mg^{2+}], 1·1 mM; glucose, 10·3 mM. When the effect on renin release of changing the [Ca^{2+}] of the incubation buffer was investigated, the volume of $CaCl_2$ (1·22 % w/v) used in the preparation of the buffer was altered as appropriate. Ca^{2+}-depleted buffers were prepared by omitting $CaCl_2$ and adding EGTA to a final concentration of 5 mM. The series of buffer [Ca^{2+}] used was 9·2 mM, 4·6 mM, 2·3 mM (control), 1·2 mM, zero, and zero [Ca^{2+}] + 5 mM-EGTA. All other constituents of the basic buffer

remained unchanged. In experiments requiring manipulation of buffer [K^+] the volume of KCl (1·15% w/v) used was altered. The series of buffer [K^+] used was 1·0 mM, 5·7 mM (control), 11·0 mM and 20·0 mM. In a further series of experiments the same range of buffer [K^+] was studied but using Ca^{2+}-depleted media (zero [Ca^{2+}] + 5 mM-EGTA). In all cases the osmolarity of the buffer was kept within the range 280–300 mosmol/l, if necessary by the addition of 0·4% mannitol. The minor differences in [Cl^-] between some buffers and the presence of mannitol in only certain buffers does not *per se* affect the secretion of active and inactive renin by rabbit kidney cortex slices (Munday *et al.* 1982).

Methods for measuring active and inactive renin have also been published elsewhere (Richards, Lush, Noble & Munday, 1981*a*). Inactive renin can only be measured after activation *in vitro*. This was achieved by acidification. Samples were dialysed against pH 2·8 glycine–HCl buffer (170 mM) for 24 h and then re-dialysed to pH 7·4 against a phosphate buffer (175 mM) for a further 24 h. Subsequent renin assay yielded a value for total renin concentration (active + inactive). Active renin alone was estimated in samples which had been dialysed against pH 7·4 buffer for 48 h. Inactive renin was therefore determined as the difference between these two sets of measurements. Active and activated renins were assayed by radioimmunoassay of generated angiotensin I (Ang I) (Stockigt, Collins & Biglieri, 1971) after incubation of dialysed samples with excess sheep renin substrate (Skinner, 1967). Results for renin estimations are expressed as renin activity released per milligram wet weight tissue (ng Ang I/h . mg) during the 90 min slice incubation.

It has been suggested that reports of inactive renins derived from species other than man may be artifactual (Sealey *et al.* 1980). Some further aspects of the assay protocols described above are therefore reviewed here. We have used acidification to pH 2·8 in order to activate inactive renin. The increased rate of Ang I generation after acid dialysis, it is argued, might conceivably result from activation of an acid protease, such as cathepsin D. As all incubations of active and activated renin with substrate were, in our studies, carried out at pH 7·4, this is very unlikely. It is, of course, a potential problem for those workers who use pH 5·5 for incubation of renin with its substrate.

Studies of plasma inactive renin can theoretically be criticized on the basis that the low-pH dialysis used to activate inactive renin may partially destroy renin substrate. Samples treated with acid and those dialysed at pH 7·4 would therefore be incubated with differing amounts of substrate. It has also been suggested that acid dialysis modifies the structure of renin substrate and that this explains the subsequent demonstration of 'inactive renin' (Gallagher, Laragh, Atlas & Sealey, 1980). Such considerations are clearly not relevant to the present study. Kidney slice supernatants do not contain significant amounts of renin substrate. Equal amounts of exogenous sheep renin substrate were added to the samples dialysed at pH 2·8 and those dialysed at pH 7·4 after the dialysis protocols had been completed.

A further suggestion that has been advanced is that acid dialysis destroys angiotensinases and therefore a larger proportion of generated Ang I survives the incubation of renin with its substrate. We evaluated this in rabbit plasma samples by adding a known amount of Ang I (25 ng/ml) to aliquots processed through each of the dialysis protocols. The percentage recovery of Ang I after incubation for 8 h at 37 °C was 85·7 ± 4·2% for samples dialysed at pH 2·8 and 82·7 ± 3·1% for those dialysed at pH 7·4 ($n = 5$). For routine measurements of renin activity 3 h incubations were used. The increase in renin activity following acidification of rabbit plasma samples cannot then be attributed to alterations in angiotensinase activity. As plasma enzymes washed out of slices would be the most likely source of peptidases in slice supernatants, we feel this generalization can be extended to *in vitro* studies as well.

Statistical analysis of results

The renin activity in each slice supernatant either with or without acidification was compared using a non-parametric paired test, the Wilcoxon signed-rank test. For analysis of data obtained for percentage changes in renin activity, a paired Student's *t* test was used. Linear regression analysis was used to relate renin concentration to ionic concentrations of the incubation buffers.

RESULTS

Effect of buffer [Ca^{2+}] on release of active and inactive renins

In Table 1 results are shown for release of active and inactive renin during incubation of kidney cortex slices in buffers with differing [Ca^{2+}]. Release of active renin is also shown as a percentage, taking the control in each experiment (unmodified buffer) as 100%. In addition, data for inactive renin are presented as a percentage of total renin released, in order to illustrate situations where a change occurred in the relative, as well as in the absolute, amounts of the two renins secreted.

When [Ca^{2+}] was reduced from the control level (2·3 mM) to 1·2 mM or zero, there were no significant changes in release of active renin unless EGTA (5 mM) was added to the incubation medium. In this case an increase occurred from $8·2 \pm 2·9$ ng Ang I/h . mg to $23·5 \pm 7·2$ ng Ang I/hr . mg ($P < 0·001$), a mean change of 167·1%. Increasing [Ca^{2+}] to 9·2 mM suppressed active renin release to $1·6 \pm 0·6$ ng Ang I/h . mg. Thus, Ca^{2+} depletion, with EGTA present, stimulated release of active renin by rabbit kidney cortex slices, whilst increasing [Ca^{2+}] suppressed it. For the relationship between release of active renin and [Ca^{2+}] the correlation coefficient (r) was −0·94.

A broadly similar pattern of response was observed for inactive renin except that the changes were quantitatively more marked. Release of inactive renin increased from $0·9 \pm 0·4$ ng Ang I/h . mg (control) to $9·8 \pm 1·7$ ng Ang I/h . mg ($P < 0·05$) in the absence of Ca^{2+} (EGTA added) and decreased to $0·3 \pm 0·1$ ng Ang I/h . mg ($P < 0·05$) when [Ca^{2+}] was raised to 9·2 mM (Table 1). Thus, instead of the nearly 3-fold increase in release of active renin in media containing EGTA, inactive renin increased by about 10-fold.

In further experiments in which slices were incubated in media containing 2·4 mM-Ca^{2+} plus 5 mM-EGTA, release of active and inactive renins was similar to control rates. This suggests that EGTA *per se* had no direct effect on renin secretion. The enhanced secretion of renins in the presence of EGTA was therefore attributed to a reduction in extracellular [Ca^{2+}].

The relative changes in the secretion of the two forms of renin are most clearly shown by expressing inactive renin as a percentage of total renin released. In control media, inactive renin formed $17·4 \pm 2·8$% of total renin (Table 1). When Ca^{2+} was omitted from the incubation media this increased to $34·5 \pm 6·2$% (no EGTA) or $34·9 \pm 6·0$% (EGTA present). In high [Ca^{2+}] media (9·2 mM) inactive renin formed only $8·3 \pm 1·3$% of the mixture of renins released. An inverse linear relationship thus existed between the proportion of total renin released which was in the inactive form and [Ca^{2+}] ($r = -0·96$). It may be significant that, in the absence of Ca^{2+} ions, the proportion of inactive renin released by slices closely resembles that found in extracts of kidney cortex. Homogenates of non-incubated slices contained $38·1 \pm 3·9$% of their renin in the inactive form ($n = 10$).

In order to check whether incubating slices in low [Ca^{2+}] media was merely changing renin release by altering cell water content, a group of cortex slices was weighed before and after a 90 min incubation in either the full Krebs buffer (control) or Ca^{2+}-depleted buffer (EGTA added). Results were as follows: in the control,

TABLE 1. Effect of $[Ca^{2+}]$ on the release of active and inactive renin by rabbit kidney cortex slices

$[Ca^{2+}]$ (mM) ...	0+EGTA	0	1·2	2·3 (control)	4·6	9·2
Active renin (ng Ang I/h . mg wet weight)	23·5±7·2***	9·9±2·7	9·0±1·3	8·2±2·9	4·4±1·4	1·6±0·6**
Active renin (% control)	267·1±58·4**	156·0±23·8*	149·0±8·7***	100	82·8±21·7	28·4±5·0***
Inactive renin (ng Ang I/h . mg wet weight)	9·8±1·7*	5·3±0·7*	2·5±0·6	0·9±0·4	0·7±0·5	0·3±0·1*
Inactive renin (% of total released)	34·9±6·0*	34·5±6·2*	27·7±6·5	17·4±2·8	9·6±1·5	8·3±1·3*

Results are means±S.E. of the mean ($n = 10$). Significance of differences: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ for paired comparison with control ($[Ca^{2+}] = 2.3$ mM).

TABLE 2. Effect of changing $[K^+]$ in the presence of 2·3 mM-Ca^{2+} (control) and in Ca^{2+}-depleted media (zero Ca^{2+} + 5 mM-EGTA) on release of active and inactive renin by rabbit kidney cortex slices

$[K^+]$ (mM) ...		1·0	5·7 (control)	11·0	20·0
Active renin (ng Ang I/h . mg wet weight)	Control $[Ca^{2+}]$	8·7±1·4	7·9±1·7	5·5±0·9	2·1±0·6**
	Ca^{2+}-depletion	4·8±0·8*†	23·6±5·7***	20·0±4·0***	24·7±2·7***
Active renin (% control)	Control $[Ca^{2+}]$	113·4±14·8	100	79·3±18·1	26·7±13·5***
	Ca^{2+}-depletion	74·1±14·2†	300·8±65·4**	373·7±83·5**	341·7±82·6**
Inactive renin (ng Ang I/h . mg wet weight)	Control $[Ca^{2+}]$	2·6±0·5**	1·1±0·4	0·3±0·1**	0·2±0·1**
	Ca^{2+}-depletion	0·8±0·3††	10·1±2·3**	12·7±2·4**	13·3±2·8***
Inactive renin (% of total released)	Control $[Ca^{2+}]$	35·4±6·4*	16·5±2·0	8·5±2·9*	6·2±2·2*
	Ca^{2+}-depletion	29·3±3·6*	24·8±3·1*	30·3±4·8*	39·2±4·8**

Results are means±S.E. of the mean ($n = 10$). Significance of differences: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ for paired comparison with control ($[K^+] = 5.7$ mM; $[Ca^{2+}] = 2.3$ mM). † $P < 0.01$; †† $P < 0.001$ for paired comparison with Ca^{2+}-depleted control ($[K^+] = 5.7$ mM; zero Ca^{2+} + 5 mM-EGTA).

pre-incubation weight was 87·5 ± 8·3 mg, post-incubation weight 94·8 ± 10·3 mg ($n = 5$); in the Ca^{2+}-depletion experiment, pre-incubation weight was 87·4 ± 6·9 mg, post-incubation weight 90·6 ± 8·5 mg ($n = 5$). No significant changes in slice weight occurred during incubation in either medium.

Active renin released during a 90 min incubation in control Krebs buffer was approximately 8% of that extractable from non-incubated slices.

Effect of buffer [K^+] on release of active and inactive renins

The effect of changing [K^+] in the presence of Ca^{2+} (2·3 mM) will first be considered. It is thought that increasing [K^+] depolarizes the juxtaglomerular cells (Fishman, 1976; Fray, 1978). However, there is no evidence to suggest how reducing [K^+] may alter the membrane potential of these cells. For normal Krebs buffer [K^+] is 5·7 mM. The effect on renin release of incubating rabbit kidney cortex slices in buffers with differing [K^+] is shown in Table 2.

Reducing [K^+] to 1·0 mM did not significantly alter release of active renin from the control rate of 7·9 ± 1·7 ng Ang I/h . mg. When [K^+] was increased to 20·0 mM release of active renin was reduced to 2·1 ± 0·6 ng Ang I/h . mg ($P < 0·01$), a mean decrease of 73·3%. Release of inactive renin did not, however, follow an exactly similar pattern. Reducing [K^+] to 1·0 mM increased inactive renin release from 1·1 ± 0·4 ng Ang I/h . mg (control) to 2·6 ± 0·5 ng Ang I/h . mg ($P < 0·01$). This is in contrast to the corresponding absence of change in active renin. When [K^+] was raised to 20·0 mM, release of inactive renin was reduced to 0·2 ± 0·1 ng Ang I/h . mg ($P < 0·01$), a more marked suppression than for active renin.

The relative changes in the two forms of renin are more clearly shown by the data expressing inactive renin as a percentage of total renin (Table 2). Incubating slices in control buffers released renin of which 16·5 ± 2·0% was inactive. In high [K^+] buffer (20·0 mM) this fell to 6·2 ± 2·2%, whereas in low [K^+] buffer (1·0 mM) 35·4 ± 6·4% of total renin was inactive. The latter value is similar to the proportion of inactive renin in homogenates of non-incubated slices which, for this group of experiments, was 37·7 ± 4·1%. Analogies are apparent between this situation and the effect of reducing [Ca^{2+}] to zero either with or without EGTA present (Table 1).

Therefore, depolarizing kidney cortex slices with high [K^+] media reduces the secretion of both forms of renin, but the effect on inactive renin is more marked than for active renin. Low [K^+] buffers appear to increase release of inactive renin but have no effect on active renin. A possible mechanism for these changes would be that they were mediated by changes in Ca^{2+} flux across the juxtaglomerular cells: depolarization by high [K^+] would probably be accompanied by increased influx of Ca^{2+} ions and the effects of low [K^+] media possibly by decreased Ca^{2+} influx. This hypothesis was investigated further in experiments described in the next section.

Role of Ca^{2+} ions in [K^+]-mediated changes in release of active and inactive renin

A series of buffers was prepared with identical [K^+] values to those described in the previous section but omitting Ca^{2+} and adding EGTA (5 mM). Results for renin release are shown in Table 2, where they can be directly compared with the results for the equivalent incubation media containing Ca^{2+}.

Release of active renin increased by 200% in Ca^{2+}-depleted media containing

5·7 mM-K^+. This is comparable to the data shown in Table 1. The elevated secretion rate was not further altered by increasing the buffer [K^+]. In other words, the inhibitory effect of depolarizing media on release of active renin was lost when Ca^{2+} ions were absent. This suggests that influx of Ca^{2+} ions was responsible for the inhibition of the release of active renin in high [K^+] buffers. In Ca^{2+}-depleted media, mechanisms controlling secretion of active renin did not respond to an increase in [K^+]. But, in low [K^+], Ca^{2+}-depleted buffers, release of active renin was suppressed below control levels. This is in contrast to low [K^+] in the presence of Ca^{2+} ions, which did not alter the release of active renin.

Release of inactive renin was again increased about 9-fold by Ca^{2+} depletion in 5·7 mM-K^+ buffers, a similar response to that shown in Table 1. In common with the results for active renin, the inhibitory effect of high [K^+] buffers on release of inactive renin was lost in the absence of Ca^{2+} ions. It is concluded that influx of Ca^{2+} ions mediates the inhibitory response to depolarization for inactive as well as active renin. However, whereas low [K^+] stimulated release of inactive renin in the presence of Ca^{2+} ions, this response was abolished in the absence of Ca^{2+} ions. Thus, low [K^+] media negated the stimulatory effect of low [Ca^{2+}] media on the release of inactive renin. One interpretation of these data is that exposure to 1·0 mM-K^+ buffer makes the juxtaglomerular cell membrane less permeable and so Ca^{2+} efflux into a Ca^{2+}-depleted environment is inhibited. Nevertheless the complete inhibition by low [K^+] of the stimulation by low [Ca^{2+}] of the release of inactive renin seems a surprising observation.

Although there was a more than 10-fold difference in the absolute levels of inactive renin released in low [Ca^{2+}]/low [K^+] buffer and in the low [Ca^{2+}]/normal [K^+] buffer, inactive renin expressed as a percentage of total renin release was similar in the two cases (29·3 ± 3·6 % and 24·8 ± 3·1 % respectively).

Thus, the decreased secretion of both active and inactive renin in depolarizing (high [K^+]) media can be conveniently attributed to increased influx of Ca^{2+} ions. Low [K^+] media, in the presence of Ca^{2+} ions, do not significantly alter release of active renin but increase the output of inactive renin. This change might be associated with a decrease in the influx of Ca^{2+} ions. It is less easy to envisage, however, why low [K^+] media should be so effective in suppressing release of both forms of renin in the absence of Ca^{2+} ions.

DISCUSSION

The data reported here on release of active renin following exposure of kidney cortex slices to low [Ca^{2+}] confirm results published from other laboratories using a range of *in vitro* preparations (Van Dongen & Peart, 1974; Fray, 1977; Ettienne & Fray, 1979; Baumbach & Skøtt, 1981; Churchill, 1981; Park *et al.* 1981). Table 1 shows that omission of Ca^{2+} ions from the incubation buffer bathing rabbit kidney cortex slices, and depletion of the tissue extracellular space with 5 mM-EGTA, increases release of active renin by 167·1 % above control. This supports the concept that a reduction in intracellular [Ca^{2+}] of the juxtaglomerular cells results in stimulation of the release of active renin. Incubating slices in depolarizing media in which [K^+] was increased from 5·7 mM (control) to 20 mM reduced secretion of active

renin but, in the absence of Ca^{2+} ions, raised $[K^+]$ ceased to have such an effect. It is envisaged that exposure to high $[K^+]$ opens voltage-sensitive Ca^{2+} channels and therefore increases the influx of Ca^{2+} ions, which in turn inhibits release of active renin.

The possibility that Ca^{2+} ions regulate release of inactive renin has not previously been investigated. Ca^{2+}-depleted media stimulated (Table 1) and depolarizing (high $[K^+]$) media inhibited (Table 2) release of inactive renin by the kidney cortex slices. This suggests that release of inactive renin is also regulated by voltage-sensitive Ca^{2+} channels. Although the results for active and inactive renin are qualitatively similar, they are not quantitatively equivalent. This is shown by the data in which inactive renin is expressed as a percentage of total renin released. Under control incubation conditions $17{\cdot}4 \pm 2{\cdot}8\,\%$ of total renin was inactive. This increased to $34{\cdot}9 \pm 6{\cdot}0\,\%$ ($P < 0{\cdot}05$) following Ca^{2+} depletion (Table 1). Depolarization with 20 mM-K^+ was accompanied by a change from $16{\cdot}5 \pm 2{\cdot}0\,\%$ (control) to $6{\cdot}2 \pm 2{\cdot}2\,\%$ of total renin in the inactive form ($P < 0{\cdot}05$) (Table 2).

Responses of renin release mechanisms to high $[Ca^{2+}]$ have been less widely reported. An increase in $[Ca^{2+}]$ from 2·3 mM (control) to 9·2 mM meant that release of active renin was reduced by 71·6%. Broadly similar results were obtained by Fray & Park (1979) from a study using isolated perfused rat kidneys. In the present study release of inactive renin was reduced more markedly than that of active renin by high $[Ca^{2+}]$. Inactive renin formed only $8{\cdot}3 \pm 1{\cdot}3\,\%$ of total renin when $[Ca^{2+}]$ was 9·2 mM compared with $17{\cdot}4 \pm 2{\cdot}8\,\%$ in the control situation ($P < 0{\cdot}05$) (Table 1). These results again fit the hypothesis that increased influx of Ca^{2+} ions inhibits release of both active and inactive renin.

It has been proposed that many stimuli for renin release, particularly those haemodynamic stimuli acting via the intrarenal stretch receptor, have their effect by hyperpolarizing the juxtaglomerular cells, decreasing their permeability to Ca^{2+} ions and possibly increasing the rate of Ca^{2+} ion extrusion (Fray, 1980). Isoprenaline is considered to increase secretion of active renin by such a mechanism. We have previously shown that rabbit kidney cortex slices respond to isoprenaline with increased secretion of active renin but no change in secretion of the inactive form (Richards *et al.* 1981*b*). It does not seem possible to cause hyperpolarization of the juxtaglomerular cells simply by manipulating buffer $[K^+]$. Fishman (1976), in a study using micro-electrode recordings of juxtaglomerular cell membrane potentials, only reported the depolarizing effects of raising external $[K^+]$, although it was shown that the cells could be hyperpolarized by adrenaline. The effect of low $[K^+]$ was not reported. The juxtaglomerular cells are considered to be modified smooth muscle cells. Low external $[K^+]$ does not change, or may even reduce, the membrane potential of other types of smooth muscle (Johansson & Somlyo, 1980; Casteels, 1981). Ions other than K^+ may contribute to the membrane diffusion potential and electrogenic active transport mechanisms may also affect the smooth muscle resting potential under low $[K^+]$ conditions.

In this context, the results from the present experiments, in which K^+ was reduced from 5·7 mM (control) to 1·0 mM, are difficult to explain. In the presence of 2·3 mM-Ca^{2+}, low $[K^+]$ media failed to alter the release of active renin but that of inactive renin increased by 135% (Table 2). As discussed previously, Ca^{2+} depletion using EGTA with normal $[K^+]$ was accompanied by a 3-fold increase in release of active renin and

a 10-fold increase in release of inactive renin. Low (1·0 mM) [K^+] abolished the effect of Ca^{2+} depletion and reduced secretion of both forms of renin to below control levels (Table 2). An interpretation of these results is that 1·0 mM-K^+ rendered the juxtaglomerular cells impermeable to Ca^{2+} ions and that, even in the face of Ca^{2+} depletion, no efflux of Ca^{2+} ions could occur. Release of both forms of renin was therefore close to control levels. What the mechanism for such a change might be cannot be defined currently. Alternative explanations of the data are also possible.

In a study carried out using rat renal cortex slices, Churchill & Churchill (1980) found that reducing [K^+] in incubation media suppressed renin release. Furthermore, low [K^+] did not oppose the effect on renin release of Ca^{2+} depletion using 2 mM-EGTA. A possible reason for the disparity between these observations and our own may lie in the species used for the work. In another experimental situation – the effect of changing [Na^+] bathing kidney cortex slices – we have already noted differences between the reported responses in the rat and in other experimental animals (Munday *et al.* 1982). This might be linked to reports that the rat kidney, in contrast to the kidney of other species such as human, dog, pig, sheep and rabbit, may not secrete an inactive form of renin (Nakane, Nakane, Corvol & Menard, 1980; de Keijzer, Provoost & Derkx, 1982). This is, however, contentious and other authors have reported that inactive renin is present in rat plasma (Barrett, Eggena & Sambhi, 1981; Gillies, Morgan & Fitzgibbon, 1982).

The data in this paper show that there is a degree of autonomy between secretory control mechanisms for active and inactive renin. In several instances there were clear quantitative differences in response to experimental manipulations. However, none of these was as dramatic as previously reported changes induced by a reduction in incubation buffer [Na^+]. In this case, active renin increased whilst release of inactive renin was abolished (Munday *et al.* 1982). This might suggest that reduction in the influx of Na^+ ions could increase conversion of inactive renin to the active form, or selectively stimulate release of active renin. Such observations provide a basis for an alternative explanation of the results in the present paper.

Juxtaglomerular granular cells are modified vascular smooth muscle cells. Na^+ ions carry inward current during spike discharges in arterial smooth muscle although Ca^{2+} ions can also carry fast inward current to some extent (Keatinge & Harman, 1980). By analogy, changes in intracellular [Na^+], in addition to [Ca^{2+}], could be important in regulating renin secretion. Reducing extracellular [Ca^{2+}] will tend to increase the permeability of the cell membrane. In consequence, intracellular [Na^+] may rise. On the basis of the hypothesis outlined above this may inhibit conversion of inactive renin to the active form within a storage pool. Subsequent exposure to renin-releasing stimuli would therefore result in relatively greater increases in the release of inactive than active renin. This was indeed observed when [Ca^{2+}] of the medium bathing the kidney slices was reduced (Table 1).

A possible further clue to the intracellular mechanisms which regulate release of active and inactive renin is provided by data reported here. Extracts of rabbit kidney yield a mixture of renins of which approximately 35% is inactive (Richards *et al.* 1981*b*; Munday *et al.* 1982) (Tables 1 and 2). Under conditions where Ca^{2+} was omitted from the incubation buffer, with or without EGTA present, or in low [K^+] buffers, the slices secreted a mixture of renins which was very similar in composition to that

identified in slice homogenates. This suggests to us that, under these circumstances, renins are released in a relatively uncontrolled way. An increase in intracellular [Ca^{2+}] brings into action inhibitory mechanisms which affect release of inactive renin more markedly than that of active renin. We have so far failed to identify any circumstance in which release of inactive renin is selectively stimulated (that is, in which more than 35% of renin released is in the inactive form). This generalization applies both to *in* vitro studies using kidney cortex slices (Richards *et al.* 1981*b*; Munday *et al.* 1982) and to *in vivo* studies of rabbit plasma renin (Grace, Munday, Noble & Richards, 1979; Richards, Grace, Noble & Munday, 1979; Richards *et al.* 1981*a*, *b*).

The demonstration in this paper that active and inactive renin are, at least to a degree, independently controlled provides further support for the idea that activation of inactive renin before secretion, or differential release of the two forms from storage pools, provides a new locus for regulation of the renin–angiotensin system.

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