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STUDIES ON THE MECHANISM OF RENIN RELEASE FROM RAT KIDNEY SLICES: CALCIUM, SODIUM AND METABOLIC INHIBITION

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SUMMARY

1. The coincident release of renin and lactic dehydrogenase (LDH) from rat renal cortical tissue slices was studied during calcium depletion, metabolic inhibition, and with the addition of ouabain (1 mM) to the incubation medium.

2. The results indicate that although LDH accumulated in the medium during incubation, the pattern was dissimilar to that of renin. Ouabain significantly inhibited renin release in calcium containing medium, but had no effect on LDH release. Renin release was potentiated in calcium 'free' media, while calcium depletion reduced the release of LDH.

3. The addition of potassium cyanide (2 mM) significantly inhibited the release of renin from these tissue slices. Cyanide was ineffective, however, when administered in calcium 'free' medium.

4. At reduced incubation temperatures (5 °C) the release of both renin and LDH were significantly reduced.

5. Medium sodium depletion caused a significant inhibition of renin release. The simultaneous removal of calcium from the medium did not restore renin release to control levels.

6. These results are not consistent with the hypothesis that spontaneous renin release during calcium depletion and metabolic inhibition is a result of cell enlargement and increased membrane permeability. On the other hand, the *in vitro* release of renin during these experiments appeared to be inversely related to the intracellular concentration of calcium.

INTRODUCTION

The rate of renin release from the juxtaglomerular cells of the renal cortex is believed to be controlled by a number of factors. Some of those currently implicated are: renal perfusion pressure, sodium load and/or concentration at the macula densa, renal sympathetic tone, and plasma catecholamine level (Davis & Freeman, 1976). The relative physiological importance of these factors and the mechanism(s) through which they operate are unknown.

Calcium has been shown to couple the processes of stimulation and secretion in a number of humoral systems (Rubin, 1974). Recently, Lester & Rubin (1977) studied the role of calcium in renin secretion in the isolated, perfused cat kidney, concluding that increases in intracellular calcium may induce renin release. However, Baumbach

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& Leyssac (1977) have reasoned from their studies with isolated, perfused glomeruli that calcium stimulated exocytosis is not the mechanism responsible for renin control. They found an inverse relationship between intracellular calcium and renin release and suggested that calcium may alter renin release through regulating membrane permeability or by acting as a second messenger in the regulation of cell volume. The recent work of other investigators supports this inhibitory role of calcium in the regulation of renin release (Fynn, Onomakpome & Peart, 1977; Peart, Quesada & Tenyi, 1977; Park & Malvin, 1978). Harada, Lester & Rubin (1979) have suggested the stimulatory effects of calcium deprivation on renin secretion are due to the action of calcium on membrane permeability, and result in spontaneous secretion which occurs by a different mechanism than evoked renin release.

The experiments in this study were designed to clarify the relationship between calcium, membrane permeability and renin secretion. Also the effect of medium sodium depletion and metabolic inhibition were studied to clarify the mechanism of ouabain inhibition of renin release.

METHODS

These studies on the control of renin release *in vitro* utilized rat renal cortical tissue slices. Sprague-Dawley rats, 200–400 g, were anaesthetized with ether, their kidneys excised and decapsulated. Four thin cortical slices were prepared from each kidney using a razor blade as described previously (Lyons & Churchill, 1974). No more than 20 min elapsed between the time the rats were killed and the tissue slices were incubated. During this period the tissue was maintained at 5 °C.

The slices were rinsed with 150 mM-sodium chloride, blotted, and placed in preweighed 25 ml. Erlenmeyer flasks which contained 10 ml. incubation medium. The flasks were stoppered and placed in an oscillating incubator at 37 °C. At 30 min periods, a sample of the incubation medium was withdrawn from each flask and centrifuged at 4 °C. The supernatant was then withdrawn and frozen for later assay.

The incubation medium consisted of a physiological salt solution of the following composition (mM): sodium chloride, 123·7; sodium bicarbonate, 19·7; potassium chloride, 4·9; calcium chloride, 2·65; potassium bicarbonate, 1·2; magnesium sulphate, 0·8 and 0·2 g% of both glucose and bovine serum albumin (United States Biochemical Corp.). The medium was maintained at pH 7·4 by equilibration with carbon dioxide and 95% oxygen. In experiments with calcium 'free' medium, the calcium chloride was replaced with 5·3 mM-sodium and 2·0 mM-EGTA (ethylene-glycol-2-(2-aminoethyl)-tetraacetic acid) was added.

The media utilized in the studies of renin secretion from tissue slices incubated in sodium-depleted medium had the following composition (mM): choline chloride, 145; potassium chloride, 6; magnesium sulphate, 0·8; calcium chloride, 2·65; Tris (tris(hydroxymethyl) aminomethane), 5; potassium calcium, 2; and 0·2 g% of both glucose and bovine albumin. Media was titrated to pH 7·4 with hydrochloric acid. The control slices in this study were incubated in similar media except the choline chloride was replaced with equimolar sodium chloride. In these series of experiments it was necessary to pre-incubate the tissue slices in their respective incubation media for 60 min at 5 °C in order to reduce the extracellular sodium level in the slices incubated in sodium 'free' medium. Also in this series the incubation flasks were equilibrated with 100% oxygen.

Following incubation the flasks and contents were dried to a constant weight at 95 °C. Weight of the solutes, obtained by drying preweighed flasks containing incubation media only, was subtracted to obtain the dry weight of the tissue in each flask.

Renin was determined by radioimmunoassay procedures. Briefly, 20 μl. of incubation medium was thawed on ice, and added to 200 μl. of previously prepared rat renin substrate (Carvalho, Shapiro, Hopper & Page, 1975). Then 8-hydroxy quinoline and dimercaprol were added to inhibit converting enzyme and angiotensinase activities. This mixture was incubated for 30 min in an oscillating water bath. Samples were then removed and analysed to determine angiotensin

I (A-I) generated during incubation. The concentration of A-I was determined by standard radioimmunoassay techniques using antisera to synthetic A-I produced by the method of Skowsky & Fisher (1975). Renin activity is expressed as ng.angiotensin I/hr substrate incubation.mg. tissue dry weight or ng.A-I/hr.mg.

The activity of lactate dehydrogenase (LDH) in the incubation media was determined by means of a commercially available kit (Sigma Chem. CO.). LDH activity is expressed as B-B units/mg dry weight of tissue. A B-B unit is the amount of LDH required to reduce $4{\cdot}8 \times 10^{-4}$ μmole pyruvate/min at 25 °C.

The results are presented as the mean with variance indicated as standard error of the mean. The significance was assessed by the Student's t test.

RESULTS

The effect of ouabain and calcium depletion on renin release and on cell permeability. The rate of renin release from renal cortical tissue slices incubated in control and ouabain (1 mM) containing medium are presented in Fig. 1, and that released from slices incubated in calcium 'free' and ouabain (1 mM) containing calcium 'free' medium in Fig. 2. The total amounts of renin found in the media at the end of 30, 60 and 90 min incubation are plotted in these Figures. The linearity of the relationships indicates that the rates of release were almost constant during the control situation.

In the presence of ouabain the release of renin from kidney slices was virtually stopped (Fig. 1). With the omission of calcium from the medium the rate of renin release was significantly elevated ($P > 0{\cdot}01$) to almost twice the control level (cf. Figs. 1 and 2). The addition of ouabain to this calcium 'free' medium did not significantly effect the renin release (Fig. 2).

Cell membranes are said to be destabilized in the absence of divalent cations (Rubin, 1974). Therefore, it is possible that the observed increase in renin release from tissues incubated in calcium 'free' medium was due to a non-specific loss of cytoplasmic contents. This possibility was examined by determining both renin and LDH release during these experiments. LDH, a cytoplasmic marker enzyme, has been shown to be a sensitive index for cell lysis (Segal & Levi, 1975; Chandler & Williams, 1977). The level of LDH increased progressively throughout the experiments (Figs. 3 and 4). However, the rate was not consistent with the rate of renin release. For instance, ouabain did not significantly affect the rate of LDH release. Unexpectedly, the activity of LDH was significantly decreased in medium from the tissue slices incubated in calcium 'free' medium at the 30 ($P < 0{\cdot}05$) and 90 ($P < 0{\cdot}01$) min incubation period. Although the presence of magnesium in the media may serve to stabilize the cell membrane, I cannot account for the increased stability which was observed in this situation. However, the changes occurring in the rate of renin release in response to ouabain and calcium depletion apparently are not the result of a non-specific loss of cytoplasmic contents.

Effect of sodium depletion. Despite the change in buffering system in the medium of this series of experiments, the rate of renin released was not significantly different from the control levels in the previous series. Likewise, no difference was observed in the levels of renin released into the calcium deficient medium in the two experimental series.

In the absence of sodium, renin release from renal cortical slices was virtually

arrested (Fig. 5). Unlike the situation with ouabain, this inhibition was not reversed by removing calcium from the medium (Fig. 6). Although there was a significant increase in renin released with calcium depletion from both the control and sodium depleted levels ($P < 0{\cdot}01$) the inhibition of *in vitro* renin release due to medium sodium depletion remained significant ($P < 0{\cdot}01$) with the simultaneous removal of calcium from the medium.

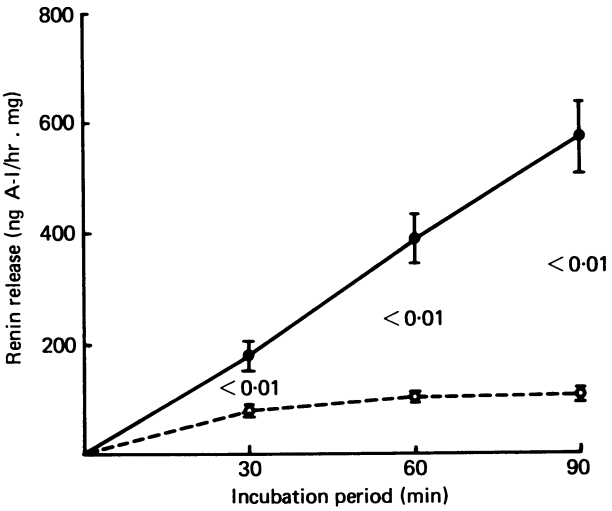

Fig. 1. Renin released from renal cortical tissue slices incubated in control, ●—●, and ouabain (1 mM) containing, ○- - -○, medium. Results are expressed as means ± S.E. ($n = 8$). P values refer to significance of difference between control and experimental group at each incubation period.

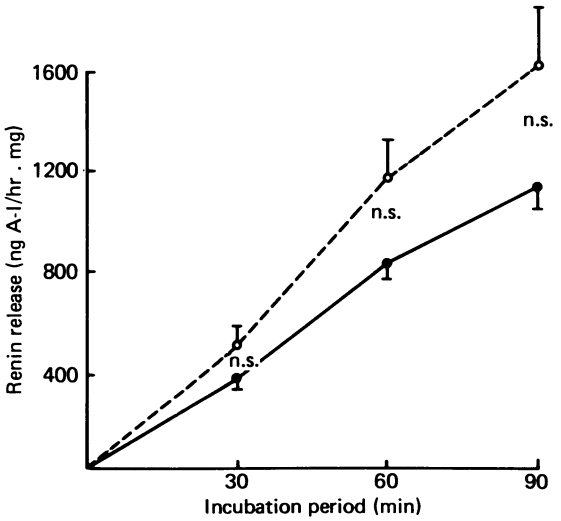

Fig. 2. Renin released from renal cortical tissue slices incubated in calcium 'free', ●—●, and ouabain (1 mM) containing calcium 'free', ○- - -○, medium. Results are expressed as means ± S.E. ($n = 8$). The difference between the groups was not significant. (n.s.) at any incubation period.

Effect of metabolic inhibition on renin release. Potassium cyanide (2·0 mM) significantly inhibited the rate of renin release from the kidney slices (Fig. 7, $P < 0{\cdot}01$). The level of this inhibition appeared to decrease after 60 min incubation. When calcium was removed from the medium containing potassium cyanide, the level of renin release was restored to control levels. The steep increase in humoral release occurring during the last sampling period from slices incubated in calcium 'free' medium containing potassium cyanide was coincident with the loss of inhibition in renin release observed with the slices incubated in potassium cyanide only.

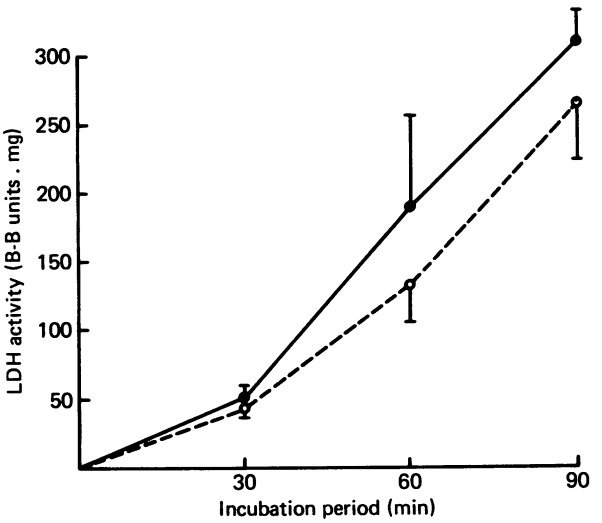

Fig. 3. Lactate dehydrogenase (LDH) released from renal cortical tissue slices incubated in control, ●—●, and ouabain (1 mM) containing, ○- - -○, medium. Results are expressed as means ± S.E. (n = 8). The difference between the groups is not significant.

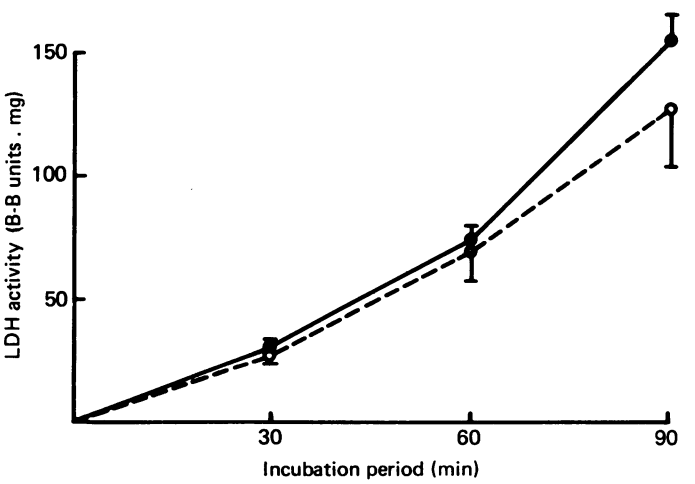

Fig. 4. Lactate dehydrogenase (LDH) released from renal cortical tissue slices incubated in calcium 'free', ●—●, and ouabain (1 mM) containing, calcium 'free', ○- - -○, medium. Results are expressed as means ± S.E. (n = 8) the difference between the groups is not significant.

The reduction of incubation temperature to 5 °C significantly decreased the concentration of renin from the levels found at 37 °C ($P < 0{\cdot}01$, cf. Table 1 and Figs. 1 and 2). At the 90 min incubation period the renin concentration in the incubation medium at this reduced temperature was 94% lower than control incubations. Neither addition of ouabain nor depletion of the medium calcium

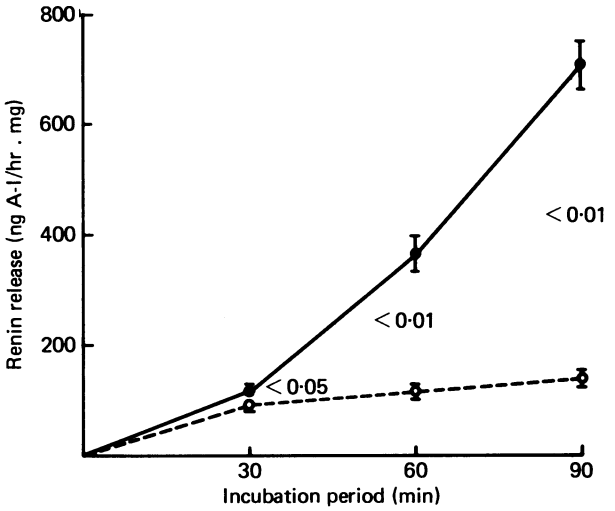

Fig. 5. Renin released from renal cortical tissue slices incubated in control, ●—●, and sodium 'free', ○- - -○, medium. Results are expressed as means ± S.E. ($n = 8$ for control and $n = 10$ for sodium 'free'). P values refer to significance of difference between the control and experimental groups of each incubation period.

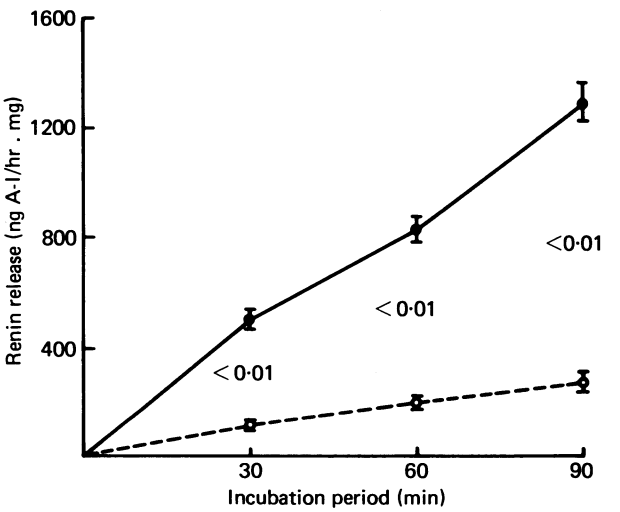

Fig. 6. Renin released from renal cortical tissue slices incubated in calcium 'free', ●—●, and sodium and calcium 'free', ○- - -○, medium. Results are expressed as means ± S.E. ($n = 8$ for calcium 'free' and $n = 10$ for sodium and calcium 'free'). P values refer to significance of difference between the two groups at each incubation period.

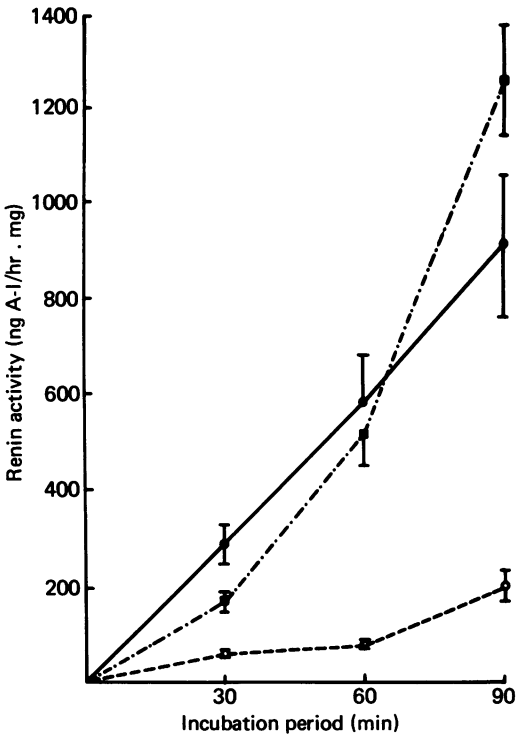

Fig. 7. Renin released from renal cortical tissue slices incubated in control ●—●, potassium cyanide (2 mM) containing, ○- - -○, and potassium cyanide (2 mM) containing, calcium 'free', ■·····■, medium. Results are expressed as means ± S.E. (n = 5 for control and n = 6 for experimental groups). Potassium cyanide significantly inhibited release ($P < 0.01$) at each incubation period, compared to release for control. No significant difference from the control was observed after calcium depletion of potassium cyanide containing medium.

TABLE 1. Effect of calcium and ouabain on renin and LDH release at reduced incubation temperature (5 °C)

	Incubation period (min)					
	30		60		90	
Medium	Renin*	LDH†	Renin*	LDH†	Renin*	LDH†
A Control (n = 8)	29·6 ± 3·0	12·4 ± 1·4	36·8 ± 2·6	15·4 ± 1·9	37·6 ± 2·9	18·1 ± 2·0
B Control + 1 mM-ouabain (n = 8)	31·8 ± 3·3	17·8 ± 1·9	39·2 ± 3·3	23·3 ± 2·5	42·8 ± 4·7	24·1 ± 2·4
C Calcium 'free' control (n = 7)	36·4 ± 4·2	16·6 ± 2·0	47·5 ± 4·8	22·8 ± 1·9	46·8 ± 5·1	23·6 ± 2·1
D Calcium 'free' control + 1 mM-ouabain (n = 8)	24·8 ± 4·0	16·2 ± 2·9	31·6 ± 4·3	20·6 ± 3·8	32·3 ± 4·6	21·4 ± 4·1

* Concentration of renin in incubation media at end of incubation period. (ng A-I/hr substrate incubation.mg tissue dry wt.)

† Concentration of lactic dehydrogenase (LDH) in incubation media at the end of incubation period (B-B units/mg tissue dry wt.).

altered the pattern of this renin release significantly. The activity of LDH present in the media was similarlt depressed at this reduced incubation temperature and was unaffected by manipulations of medium constituency in this series of experiments.

DISCUSSION

Calcium has clearly been demonstrated to act as a mediator in the release of hormones from secretory granules (Rubin, 1974). As initially proposed by Douglas (1968) and expanded extensively by Rubin (1974), calcium is believed to act as a coupler of stimulation and secretion of hormones; the concentration of calcium at some critical intracellular site being directly related to the level of humoral release. Lester & Rubin (1977) have reported that the release of renin from the granules of the juxtaglomerular cells of the renal cortex follows this universal hypothesis (Harada *et al.* 1979). However, work from several other laboratories has indicated that the concentration of intracellular calcium may be inversely related to renin release (Fynn *et al.* 1977; Peart *et al.* 1977; Park & Malvin, 1978). The secretion of renin in response to both ouabain and calcium depletion is in conflict with a direct relationship between intracellular calcium concentration and renin release.

Several reports (Baumbach & Leyssac, 1977; Harada *et al.* 1979) as well as the present study, demonstrate a significant renin stimulation in response to low extracellular calcium concentration. Since the functional integrity of cell membranes is partially dependent upon the presence of free calcium or magnesium (Rubin, 1974), the possibility had to be considered that renin release was the consequence of membrane destabilization. This would explain a high rate of spontaneous renin release found in calcium depleted media and a tendency towards higher levels of release in calcium 'free' medium containing ouabain. As assessed by monitoring the accumulation of a cytoplasmic marker enzyme, LDH, the cytoplasmic contents did increase in the medium during the incubation period. However, this rate of accumulation was not affected by either calcium or ouabain in a manner similar to renin (Figs. 3, 4).

Ouabain, while inducing the release of hormones from a number of tissues such as the adrenal medulla (Banks, 1967), neurohypophysis (Dicker, 1966), pancreas (Grodsky, 1970), and neurotransmitter release from motorneurones (Birks & Cohen, 1968), inhibits the release of renin from the kidney (Lyons & Churchill, 1974; Lyons & Churchill, 1975; Park & Malvin, 1978). Ouabain has been shown to inhibit plasma membrane sodium-potassium ATPase resulting in the equalization of intracellular and extracellular sodium concentrations (Whittam & Willis, 1963; Maude, 1969). Furthermore, ouabain inhibition of renin release is reversed when the calcium is removed from the medium as demonstrated recently by Park & Malvin (1978) and confirmed in this study. In order to confirm that ouabain was acting through inhibition of sodium-potassium ATPase inhibition, the release of renin was studied from tissue slices incubated in sodium 'free' medium. A comparison of Figs. 1 and 5 indicates that the addition of ouabain and the depletion of sodium exert nearly identical effects on renin release *in vitro*. This result is especially interesting considering the proposed role of sodium in the regulation of renin release through the

macula densa chemoreceptor (Vander, 1967; Lyons & Churchill, 1975). These studies would be evidence against that proposal. When extracellular sodium is decreased, the intracellular sodium concentration would also be expected to decrease. In contrast, incubation of tissue slices in the presence of ouabain should increase the level of intracellular sodium. Nevertheless, in both of these situations, the renin release is inhibited. The common denominator is more likely intracellular calcium rather than sodium concentration. Since media sodium depletion results in a loss of the sodium gradient across the plasma membrane, the sodium–calcium exchange diffusion which is proposed to regulate intracellular calcium concentration, should be altered as if ouabain had been present in the medium. Since the calcium and sodium 'free' media have different buffering systems and slightly different osmolalities, other factors may be responsible for the renin responses. However, this possibility is doubtful since the control release of renin in both situations is nearly identical.

The removal of calcium from the medium of tissue slices incubated in sodium 'free' medium failed to restore the renin release to control levels (Fig. 6). This may be due to the lack of sodium ions in the extracellular fluid which provide substrate for the sodium–calcium exchange diffusion. Without calcium efflux from the secretory cell, a condition necessary due to the continual loss of calcium from organelles that have a high concentration such as the mitochondria, renin release would be inhibited due to high intracellular calcium. Even with a gradient favouring calcium efflux, extracellular sodium may be a necessary condition for calcium translocation.

Baumbach, Leyssac & Skinner (1976) have reported renin release from isolated, superfused rat glomeruli is increased by metabolic inhibitors and reduced incubation temperature. This work leads again to the hypothesis that spontaneous release of renin is the result of an increase in cell size and consequent increase in cell permeability. The present work, however, indicates that metabolic inhibitors, at least potassium cyanide and reduced incubation temperature, inhibit renin release drastically. It may be that dissociation of the components of the juxtaglomerular apparatus or loss of the integrity of the tissue in some way alters the responsiveness of the juxtaglomerular cells.

The mechanism of inhibition of renin release by cyanide may be similar to that of ouabain. The inhibition induced by potassium cyanide, an inhibitor of cellular respiration (Schubert & Brill, 1968), was also entirely reversed by the depletion of medium calcium. Potassium cyanide may reduce the energy available to the transport enzyme, while ouabain directly inhibits the enzyme's activity. The loss of a transmembrane sodium gradient due to the inclusion in the media of either of these inhibitors, would result in an increase in the intracellular calcium concentration due to the loss of sodium–calcium exchange diffusion. The mechanism of reduced temperature inhibition of renin release in this system is probably more complex. It is not reversed with simultaneous calcium depletion and may depend on many factors such as membrane permeability and exocytosis mechanics.

In summary, the results from this study do not justify the hypothesis that reported increases in renin release occurring in response to calcium depletion, metabolic inhibition, and reduction in incubation temperature are due to increases in membrane permeability inciting a spontaneous loss of renin granules. Also the studies of renin

release from sodium deficient tissue slices indicate that the mechanism of ouabain induced renin inhibition may be the loss of a sodium gradient across the cell membrane.

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