Studies on the Isolation and Properties of Renin Granules from the Rat Kidney Cortex

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The present study was undertaken to isolate and investigate some physicochemical properties of renin granules from the rat kidney cortex. Two preparations of subcellular organelles were used: a primary-granule fraction, which allowed the properties of lysosomes to be compared simultaneously with those of renin granules, and a semi-purified preparation of the latter. The specific activity of renin in the primary-granule preparations was about 4-fold higher than in the original homogenate; that of the semi-purified renin-granule preparation was about 18-fold higher than in the homogenate, and consisted mainly of electron-dense granules but some mitochondria were also observed. Renin and acid phosphatase release from the primary-granule preparation was increased by lowering osmolality, by a low-molecular-weight solute (glucose) and by Triton X-100 or digitonin. Enzyme release was decreased by lowering the incubation temperature (4°C) or the presence of CaCl_2 . Renin release from the partially purified granule preparation was not affected by cyclic AMP, cyclic GMP and ATP.

Renal renin (EC 3.4.99.19) in the rat kidney cortex has been localized by means of tissue fractionation to sedimentable fragile subcellular organelles which differ in their density equilibration behaviour from other known organelles (Morris & Johnston, 1976; Morimoto *et al.*, 1972).

Limited information is available on the properties of these granules and on the mechanism of renin release at the subcellular level. Consequently a series of investigations was initiated to isolate and study certain properties of these particles. Simple and rapid procedures have been developed for the isolation of renin granules by means of differential and density-gradient centrifugation. The release of renin from the isolated granules has been investigated in response to changes in temperature, osmolality, pH and ionic composition of the incubation medium, as well as by membrane-acting agents (Triton X-100 and digitonin). The release of acid phosphatase from isolated lysosomes has also been investigated in response to these factors to compare the two particles. Additionally, the effect of ATP, cyclic AMP and cyclic GMP on renin release has been studied.

Materials and Methods

Homogenization of kidney cortex

Male Wistar rats (180–200g) were used and these had free access to water and standard laboratory

food before all experiments. Rats (two to four) were killed by cervical dislocation and the kidneys were removed, stripped of the capsule and bisected. The cortices were separated from the medulla and diluted 3–4-fold (w/v) in cold sucrose (0.3M, 4°C) and homogenized with six passes of a hand-operated loose-fitting pestle (diameter clearance 0.49mm). The homogenate was centrifuged at 300g for 60s, and the supernatant was removed. The sediment was resuspended in 0.3M-sucrose to the original volume and rehomogenized with a tighter-fitting pestle (diameter clearance 0.1mm) with six full passes and pooled with the previous supernatant.

Preparation of a primary-granule fraction

This was isolated by a modification of the procedure of Maunsbach (1964). The homogenate was centrifuged at 300g (r_{av} , 19cm) for 10min, the sediment of unbroken cells and nuclei was discarded and the supernatant was centrifuged at 260g for 10min. The resulting supernatant (8–12ml) was layered on sucrose (0.6M, 4ml) and centrifuged for 10min at 3700g for 10min. The pellet formed after centrifugation was macroscopically in two layers (Fig. 1b, upper part). The supernatant was aspirated, and about 2ml of the 0.6M-sucrose layer was aspirated and the light layer was detached from the bottom layer (primary-granule fraction) by applying a gentle tilting and swirling action on the tube and was decanted. The primary-granule fraction was washed with about 2ml of 0.6M-sucrose and resuspended in 0.6M-sucrose (4–6ml).

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Isolation of a renin-granule fraction

The primary-granule fraction (2 ml, 4 mg of protein) was applied on top of a sucrose gradient consisting of 1.6 M (10 ml) and 1.46 M (10 ml) and centrifuged for 20 min at 80 000g (r_{av} , 9.6 cm) in a 3 × 23 ml swing-out rotor on a Superspeed 40 Ultracentrifuge (MSE). The tubes were individually separated into four fractions by aspiration from top to bottom and a pellet, which was resuspended in 0.6 M-sucrose (Fig. 1b, lower part). The renin fraction (fraction 3) was diluted with 2 vol. of 0.3 M-sucrose and centrifuged at 11 000g for 10 min, and the pellet was resuspended in 0.6 M-sucrose (4 ml). Alternatively when the renin granules were used for studies on renin release, fraction 3 was diluted with 1 vol. of 0.3 M-sucrose and layered on top of 1.0 M-sucrose (4 ml) and centrifuged at 44 000g for 20 min and the pellet was resuspended in 0.6 M-sucrose to about 0.1–0.2 mg of protein/ml.

Enzyme assays

Succinate dehydrogenase [succinate-(acceptor) oxidoreductase, EC 1.3.99.1], alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) and catalase (H_2O_2 - H_2O_2 oxidoreductase, EC 1.11.1.6) were used as markers for mitochondria, plasma membranes and peroxisomes respectively and acid phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) was used as a lysosomal marker.

Renin was estimated by measuring the angiotensin I formed from homologous renin substrate prepared from the plasma of 24 h-nephrectomized rats and treated as described by Skinner (1967). The incubation system contained: kidney fraction (100 μ l), renin substrate (100 μ l), sodium phosphate buffer, pH 7.4 (600 μ l), prepared as described by Skinner (1967), EDTA (100 μ l, 0.3 M) and 2,3-dimercaptopropan-1-ol (100 μ l; 2%, v/v, in water). Incubations were carried out at 37°C and the reaction was terminated by boiling (10 min). The samples were diluted 6-fold in radioimmunoassay buffer, centrifuged to sediment coagulated proteins and the angiotensin I was estimated directly by the procedure of Boyd & Peart (1974). [Asp¹, Ile⁵]Angiotensin I (Schwarz/Mann, Wembley, Middx., U.K.) was used as a standard. Angiotensin I recovery during the incubation was determined by adding standard angiotensin I (25 ng) to the reaction mixture incubated without substrate and expressed as a percentage of control incubations without sample or substrate.

Succinate dehydrogenase was assayed by a minor modification of the procedure described by Pennington (1961), and acid phosphatase by that described by Tappel (1972) with *p*-nitrophenyl phosphate as the substrate. Alkaline phosphatase was determined by the procedure of Shibko & Tappel

(1965) and catalase by the method of Baudhuin *et al.* (1964).

Other methods

Protein was determined by the method of Lowry *et al.* (1951), with bovine plasma albumin as a standard (fraction V; Armour Pharmaceutical Co., Eastbourne, Sussex, U.K.).

Electron microscopy

The particulate fractions were diluted with 1 vol. of 0.3 M-sucrose and centrifuged at 44 000g (r_{av} , 9.9 cm) for 10 min. The pellets of subcellular organelles were fixed in 1.5% (v/v) glutaraldehyde in 0.075 M-sodium phosphate/0.6 M-sucrose buffer, pH 7.4, for 60 min at room temperature, washed once in the phosphate/sucrose buffer and post-fixed for 60 min in 1% OsO_4 , prepared by diluting a stock solution (2%, w/v, in water) with an equal volume of the buffered sucrose. The pellets were washed twice in the buffered sucrose, dehydrated through a series of aqueous ethanol solutions and embedded in Epon resin. Thin sections (0.04 μ m) were examined in a Philips 300 electron microscope.

Determination of renin and acid phosphatase release from isolated granules

Freshly prepared suspensions of the primary-granule fraction and of the renin-enriched granule preparation were centrifuged for 30 s at 300g and the aggregate-free suspensions were used. A portion (100 μ l) containing about 2 mg of protein/ml of primary-granule or renin-granule fraction (0.1–0.2 mg of protein/ml) was added in duplicate to the test solution (900 μ l) in 2 ml plastic tubes (Luckham Ltd., Burgess Hill, West Sussex, U.K.) at 4°C. The tubes were capped, the suspensions were mixed gently by inversion and subjected to the various experimental conditions as described in the Results section. On completion of these experimental procedures the tubes were centrifuged for 10 min at 4000g (r_{av} , 21 cm) at 4°C, and a portion (400 μ l) of each clear supernatant was removed and assayed for renin and acid phosphatase activity when the primary-granule fraction was used and for renin activity only when the semi-purified renin-granule preparation was used. Control samples for total activity and for the effect of the test substances on the enzyme activities were also included. When required, solutions were buffered with 10 mM-Tris (Sigma, Poole, Dorset, U.K.).

The KCl buffer consisted of KCl (140 mM), NaCl (4 mM), $CaCl_2$ (100 μ M), $MgCl_2$ (100 μ M) and Tris (10 mM), pH 6.6 (37°C). Triton X-100 and digitonin

Table 1. *Preparation of a primary-granule fraction*

(a) Distribution of renin, selected marker enzymes and protein in isolated fractions as a percentage of the total in all fractions. Recoveries are defined as a percentage of the sum of the activities in the fractions isolated (supernatant + light layer + primary-granule fraction) relative to the total activities of the unfractionated nuclear supernatant. (b) Relative specific activity, calculated by dividing the specific activity in each fraction by that in the original homogenate. The fractions were isolated as described in the Materials and Methods section. Results are the means $\pm$ s.d. for four to six experiments.

Enzymes		Fractions isolated			Recovery
		Supernatant	Light layer	Primary granule	
Renin	(a)	49.9 $\pm$ 8.0	24.8 $\pm$ 9.5	25.2 $\pm$ 5.2	95.3 $\pm$ 18.8
	(b)	0.82 $\pm$ 0.4	1.2 $\pm$ 0.3	4.7 $\pm$ 1.1	
Succinate dehydrogenase	(a)	36.8 $\pm$ 8.2	50.7 $\pm$ 9.7	12.4 $\pm$ 3.3	89.0 $\pm$ 18.1
	(b)	0.47 $\pm$ 0.1	1.9 $\pm$ 0.3	1.7 $\pm$ 0.2	
Acid phosphatase	(a)	75.4 $\pm$ 4.6	15.9 $\pm$ 4.7	8.3 $\pm$ 1.6	96.2 $\pm$ 20.6
	(b)	1.0 $\pm$ 0.3	0.72 $\pm$ 0.3	1.5 $\pm$ 0.2	
Alkaline phosphatase	(a)	71.0 $\pm$ 7.3	25.8 $\pm$ 7.4	3.0 $\pm$ 1.2	83.9 $\pm$ 18.0
	(b)	0.93 $\pm$ 0.1	1.1 $\pm$ 0.3	0.43 $\pm$ 0.1	
Protein	(a)	70.3 $\pm$ 5.2	23.4 $\pm$ 6.1	6.2 $\pm$ 1.0	83.5 $\pm$ 38.7

(BDH, Poole, Dorset, U.K.) were dissolved in 0.3 M-sucrose, and incubations were carried out at 4°C for 6 min. ATP (disodium salt from equine muscle), cyclic GMP (sodium salt) and cyclic AMP were obtained from Sigma.

Renin and acid phosphatase release were expressed as a percentage of controls frozen immediately before the incubation. Total recovery after 60 min incubation was also expressed as a similar percentage. Statistical significance between the mean values of parallel incubations was tested by Student's *t* test (one-tail). Human serum albumin was obtained from The Blood Product Laboratory, Lister Institute, Elstree, Herts., U.K., and purified bovine plasma albumin from Koch-Light Laboratories, Colnbrook, Bucks., U.K.

Results

Isolation of a primary-granule fraction

Whereas renin was found in all fractions isolated, the primary-granule preparation contained about 25% of the total renin recovered with a 4–5-fold increase in specific activity over the original homogenate (Table 1).

In contrast, the fraction contained only 3% of the alkaline phosphatase, with decreased specific activity, and 12% of the total recovered succinate dehydrogenase, with a slight increase in specific activity (1.7-fold).

Acid phosphatase was recovered mainly in the supernatant, but 8% of the total recovered was in the primary-granule preparation with a 1.6-fold increase in relative specific activity and only 6% of the protein was found in this preparation. The remainder of the protein and enzyme activities was

found in the light layer and in the supernatant fractions (Table 1).

Preparation of a renin-granule fraction

The primary-granule preparation was resolved into two distinct particulate bands and a yellow-brown pellet by gradient centrifugation (Fig. 1).

Renin was recovered mainly in fraction 3, which contained about 63% of the total and showed an 18-fold increase in specific activity over the homogenate

Fig. 1. *Isolation of renin granules by differential and density-gradient centrifugation*

Diagrammatic representation of the isolation procedure and identification of the fractions isolated: (a) before centrifugation (cross-hatched area represents original position of sample and M refers to molarity of sucrose) and (b) after centrifugation as described in text. F indicates the fractions collected and their positions in the tubes.

Fig. 2. Distribution and specific activity of renin and selected marker enzymes after density-gradient centrifugation of the primary-granule fraction

The right ordinate shows the enzyme and protein distribution (as percentage of the total recovered in the gradient; —) and the left ordinate the relative specific activity of the enzymes in each of the fractions isolated (----). The positions of the fractions (1–5) in the original tubes are illustrated in Fig. 1. Relative specific activity is expressed as described in Table 1. Results are the means for four experiments.

(Fig. 2). Succinate dehydrogenase was associated entirely with the top densest particulate band (fraction 2, Fig. 1), which contained approx. 90% of the recovered enzyme with an increase in specific activity of 2.9 (Fig. 2). In contrast, acid phosphatase, the lysosomal marker enzyme, was found predominantly in the denser regions (fractions 4 and 5, Fig. 1), with about 53% of the recovered activity and an increase in specific activity of 4.8 and 3.9 respectively (Fig. 2). Most of the protein was in fractions 2 and 3, representing 43 and 25% of the total recovered respectively.

After dilution and centrifugation of the renin-rich fraction (fraction 3 in Fig. 1), about 80% of the total renin activity could be sedimented, presumably in intact granules, without a decrease in specific activity

(Table 2). In contrast, the relative specific activity of marker enzymes was low and ranged from 0.3 to 2.0.

Electron microscopy (Plate 1)

The fraction enriched in succinate dehydrogenase (Plate 1a; fraction 2 from Fig. 2) consisted essentially of mitochondria in high purity and good morphological preservation. Some appeared swollen and damaged.

In contrast, the acid phosphatase-rich fraction (Plate 1c, fraction 5 from Fig. 2) displayed a high content of large granules with homogeneous compact electron-dense matrix and little contamination by other cytoplasmic organelles.

Although the succinate dehydrogenase- and

EXPLANATION OF PLATE I

Electron microscopy of subcellular organelles prepared by density-gradient centrifugation

(a) Mitochondria from the fraction containing highest succinate dehydrogenase activity (fraction 2 in Fig. 2) ($\times 3200$).
(b) Granular organelles from the renin-enriched fraction (fraction 3 in Fig. 2) ($\times 1200$). (c) Lysosomal-like structures from the acid phosphatase-enriched fraction (fraction 5 in Fig. 2) ($\times 17000$). Details of isolation conditions and preparation of samples for electron microscopy are given in the Materials and Methods section.

Table 2. *Relative specific activity of renin and selected marker enzymes in the renin-rich granule fraction*

The fraction was isolated by density-gradient centrifugation as described in the text. Relative specific activity is expressed as described in Table 1. Results are means $\pm$ S.D. for three to four experiments.

Enzyme	Relative specific activity
Renin	17.0 ± 3.0
Succinate dehydrogenase	0.23 ± 0.14
Alkaline phosphatase	0.40 ± 0.07
Acid phosphatase	2.0 ± 0.4
Catalase	0.76 ± 0.13

acid phosphatase-enriched fractions were morphologically homogeneous, the renin-rich fraction (Plate 1b, fraction 3 from Fig. 2) showed a heterogeneous morphology and consisted essentially of mitochondria and granular cytoplasmic granules, the latter with an electron-dense matrix.

Renin and acid phosphatase release from a primary-granule fraction

Effect of incubation on total enzyme activity. In all experiments listed below the total renin and acid

phosphatase activity after 60 min incubation was not significantly different from control samples, which were frozen immediately, and ranged from 93 to 116%.

Effect of temperature (Fig. 3a). The release of renin and acid phosphatase was decreased 3-fold and 2-fold respectively after incubation at 4°C when compared with incubation at 37°C.

Effect of osmolality (Fig. 3b). The renin and acid phosphatase release in the supernatant was increased 2–3-fold in samples incubated in 0.1 M-sucrose when compared with those incubated in 0.3 M-sucrose at the same temperature (37°C).

Effect of glucose (Fig. 3c). Incubation in glucose (0.3 M) increased the renin and acid phosphatase release 2–3-fold when compared with incubations in sucrose (0.3 M) at the same temperature (37°C). In these experiments solutions were buffered with Tris (10 mM, pH 6.6).

Effect of CaCl_2 (Fig. 3d). Incubations in sucrose (0.3 M) containing 1 mM- CaCl_2 decreased by 3-fold the renin released when compared with samples incubated under the same conditions without CaCl_2 . The release of acid phosphatase was also decreased but to a smaller extent (less than 2-fold).

Fig. 3. *Effects of various incubation conditions on renin and acid phosphatase release from a primary-granule fraction* Enzyme release after 60 min incubation is shown on the ordinate as a percentage of the total in non-incubated samples. The results are means $\pm$ S.D. for three to four parallel experiments. (a) Incubations at 37°C (□) or 4°C (■); (b) Incubation in (□) 0.3 M- and (■) 0.1 M-sucrose; (c) Incubations in 0.3 M-sucrose (□) or 0.3 M-glucose (■); (d) Incubations in 0.3 M-sucrose (□) and in 0.3 M-sucrose with 1 mM- CaCl_2 (■). The difference between the mean values of paired experiments was statistically significant ($P < 0.02$) in all cases, except for acid phosphatase (a) and (d) where the difference of the mean values in paired experiments was $P < 0.05$.

Table 3. *Effect of incubation pH on renin and acid phosphatase release from a primary-granule fraction*
Isolated granules were incubated at 37°C in 0.3M-sucrose, for 20 and 60min unbuffered or buffered with Tris/HCl. The results are means $\pm$ S.D. for three parallel experiments.

pH	Renin		Acid phosphatase	
	20min	60min	20min	60min
Unbuffered	5.6 $\pm$ 1.4	9.8 $\pm$ 6.1	20.5 $\pm$ 7.8	24.1 $\pm$ 8.6
6.6	5.1 $\pm$ 1.7	7.3 $\pm$ 2.2	22.3 $\pm$ 9.2	25.7 $\pm$ 9.0
7.2	6.7 $\pm$ 2.9	10.3 $\pm$ 4.2	28.7 $\pm$ 9.6	35.9 $\pm$ 3.5

Table 4. *Effects of incubation in buffered sucrose or KCl and albumin on renin and acid phosphatase release from a primary-granule fraction*

Isolated granules were incubated at 37°C for 20 and 60min. Solutions were buffered with Tris/HCl, pH 6.6. The results are means $\pm$ S.D. for three or four parallel experiments.

Experimental conditions	Renin		Acid phosphatase	
	20min	60min	20min	60min
0.3M-Sucrose	12.9 $\pm$ 5.3	13.6 $\pm$ 4.8	25.5 $\pm$ 7.4	27.6 $\pm$ 6.2
0.3M-Sucrose+albumin	17.8 $\pm$ 10.4	22.7 $\pm$ 12.3	27.6 $\pm$ 11.1	30.5 $\pm$ 6.5
KCl buffer	14.3 $\pm$ 10.7	19.5 $\pm$ 12.8	26.7 $\pm$ 7.9	38.8 $\pm$ 2.2
KCl buffer+albumin	27.2 $\pm$ 13.1	39.9 $\pm$ 16.0	27.0 $\pm$ 8.3	45.0 $\pm$ 5.7

Effects of incubation pH. There was no significant effect on renin release when granules were incubated in unbuffered or buffered (pH 6.6 and 7.2) sucrose solutions (0.3M) as shown in Table 3. A similar response was also observed for the release of acid phosphatase (Table 3).

Effects of incubation in 0.3M-sucrose or iso-osmolar KCl buffer with or without human serum albumin. The release of renin and of acid phosphatase after 20 and 60min was not altered by incubation in either sucrose (0.3M, pH 6.6) or KCl (Table 4). Incubations in the presence of human serum albumin (2mg/ml) resulted in a slight increase in the release of acid phosphatase when compared with parallel samples incubated without the albumin in both types of incubation medium (Table 4). The release of renin was slightly increased in samples incubated with albumin in sucrose in comparison with those without. However, when incubations were carried out in KCl buffer with albumin the renin released was about two to three times higher than in samples incubated without (Table 4). The difference between the means at both 20 and 60min incubations was statistically significant ($P < 0.05$).

Effects of Triton X-100 and digitonin. The release of renin and of acid phosphatase was proportional to the concentration of these agents in the incubation medium (Fig. 4). Low concentrations of Triton X-100 (0.006%, v/v) did not alter the enzyme released in the supernatant, but higher concentrations (0.025%, v/v) resulted in full release of renin and acid phosphatase activity.

Fig. 4. *Effect of Triton X-100 and digitonin on renin and acid phosphatase release from a primary-granule fraction*
Isolated granules were incubated at 4°C for 6min in (a) Triton X-100 (0–0.05% in 0.3M-sucrose) and (b) digitonin (0–100 μM in 0.3M-sucrose). Enzyme release is shown on the ordinate as a percentage of non-incubated controls for renin (□) and acid phosphatase (■). Results are means for three experiments.

Digitonin (10 μ M) released about 40% of the total renin activity and higher concentrations (80 μ M) resulted in complete release of the total activity to the supernatant. In contrast, 80 μ M-digitonin caused the release of only about 60% of the total acid phosphatase activity.

Renin release from the renin-enriched granule fraction

Incubation in buffered sucrose and KCl (pH 6.6) did not alter the total renin activity after incubation at 37°C for 60 min when compared with controls frozen immediately, and the recovery was essentially 100% in all experiments (mean range 73–115%).

The mean renin activity recovered in the supernatant of samples incubated in buffered 0.3 M-sucrose after 20 and 60 min was 8 and 12% of the total respectively (Table 5). In contrast, the mean renin activity from the supernatant of samples incubated in the KCl buffer after 20 and 60 min was 19 and 28% of the total respectively (Table 5). However, the difference between the means was statistically significant ($P < 0.05$) only for the 20 min values.

Effects of ATP, cyclic AMP, cyclic GMP, human serum and purified bovine plasma albumin on renin release from the renin-enriched granule fraction

The effects of these substances on renin release was investigated in both sucrose and KCl buffer.

ATP, cyclic AMP or cyclic GMP (all at 1 mM) did not change the total renin activity after 60 min incubation nor did they alter the renin released in the supernatant after 20 and 60 min incubation (Table 5). The mean values of renin recovered from the supernatant in samples incubated in 0.3 M-sucrose after 20 min incubation was 8.2%, whereas in those containing the nucleotides it ranged from 9 to 13% (Table 5). These substances did not alter the renin released in the supernatant after 20 and 60 min incubation in KCl buffer (Table 5).

Human serum albumin and purified bovine plasma

albumin (2 mg/ml) did not alter the total renin activity when compared with controls frozen immediately. However, the albumins increased 2–6-fold the renin activity recovered from the supernatants when compared with samples without the albumin in both types of incubation medium at 20 and 60 min (Table 5). This increase in recovery, at least to some extent, might represent a prevention of renin adsorption on the tubes, in view of the low protein content in this renin preparation.

Discussion

Since renin is localized near the juxtaglomerular apparatus of the kidney cortex, the purification of renin granules from a cortical homogenate is rather difficult, because of the large numbers of subcellular organelles arising from tubular structures. Although during differential centrifugation renin particles sediment mainly in the heavy-mitochondrial fraction (Morris & Johnston 1976; Morimoto *et al.*, 1972), attempts to purify these particles by subsequent sucrose-density-gradient centrifugation have only met with partial success (Morris & Johnston, 1976; Morimoto *et al.*, 1972).

Therefore, the use of the primary-granule fraction as a starting preparation for gradient centrifugation provided an advantage over a total heavy-mitochondrial fraction in view of the lower content of mitochondria, membranes and peroxisomes and of the higher initial renin content (Table 1).

Although the primary-granule fraction was also enriched in phagolysosomes (Straus, 1967; Maunbach, 1964), renin is not associated with these structures, since when this fraction was subjected to density-gradient centrifugation the distribution of renin was clearly different from that of the lysosomal marker enzyme (Fig. 2). This is in contrast with the earlier suggestion that renin was associated with lysosomes (Nustad & Rubin, 1970; Onoyama *et al.*, 1973).

Table 5. *Effects of various nucleotides on renin release from isolated granules*

Incubations were carried out at 37°C in buffered sucrose or KCl for 20 and 60 min. The results are means $\pm$ S.D. for five experiments for the controls and for three for the remainder. For further details see the text. HSA, human serum albumin; BPA, bovine plasma albumin.

Experimental condition	Percentage of renin released			
	20 min		60 min	
	Sucrose	KCl	Sucrose	KCl
Control	8.2 $\pm$ 5.7	19.2 $\pm$ 8.4	12.2 $\pm$ 10.9	27.8 $\pm$ 15.5
+ATP	13.1 $\pm$ 10.1	12.9 $\pm$ 4.8	12.5 $\pm$ 7.8	13.5 $\pm$ 5.5
+Cyclic AMP	10.3 $\pm$ 7.3	13.6 $\pm$ 3.7	12.8 $\pm$ 9.4	19.6 $\pm$ 8.0
+Cyclic GMP	9.1 $\pm$ 4.5	14.9 $\pm$ 2.7	15.0 $\pm$ 6.6	20.1 $\pm$ 3.6
+HSA	31.8 $\pm$ 9.4	62.3 $\pm$ 11.5	42.5 $\pm$ 14.4	75.1 $\pm$ 21.7
+BPA	42.8 $\pm$ 4.4	47.6 $\pm$ 14.3	77.2 $\pm$ 8.8	64.6 $\pm$ 8.0

Renin granules were partially separated from mitochondria and lysosomes by the density-gradient-centrifugation procedure. However, whereas mitochondrial and lysosomal fractions were morphologically homogeneous, the renin-granular fractions contained mitochondria in addition to large lysosomal-like structures (Plate 1).

The primary-granule fraction was used to study the release of renin and of acid phosphatase in order to compare the properties of lysosomes with those of renin granules. Both renin and acid phosphatase were apparently stable against lysosomal breakdown and this was expected, since lysosomal proteolysis by isolated renal lysosomes requires an acid pH (5.0) and ATP for optimal activity (Mego, 1971; Mego *et al.*, 1972).

Studies on the release of renin and of acid phosphatase from the primary-granule fraction indicated that renin granules are similar to lysosomes in responses to changes in temperature, osmolality, to a low-molecular-weight solute such as glucose (Fig. 3) and pH (Table 3).

Badenoch-Jones & Baum (1974) have found that renal lysosomes are unstable in low-molecular-weight solutes (glycerol and urea) as described here for glucose (Fig. 3). The response of lysosomes to changes in pH has previously been investigated by Shibko & Tappel (1965) and Shibko *et al.* (1965) and their results are in agreement with those of the present study (Table 3).

It is relevant that renin granules and lysosomes are similar to chromaffin granules in their response to changes in temperature and low-molecular-weight solutes (Perlam, 1976) and resemble insulin granules (Coore *et al.*, 1969) and prolactin granules (Gianmatteo *et al.*, 1975) in their responses to changes in pH.

Like other subcellular organelles, such as liver lysosomes (Ignarro, 1973), renin granules and renal lysosomes were equally stable in a medium more representative of the intracellular ionic environment (KCl buffer) in comparison with 0.3M-sucrose. The effects of CaCl_2 (Fig. 3d) on the release of renin are of particular interest, since it has been suggested that changes in calcium flux play a central role in the modulation of renin release (Peart *et al.*, 1977; Fynn *et al.*, 1977), as well as in other secretory systems (Rubin, 1970; Smith & Ignarro, 1975).

The subcellular mechanism of the action of calcium is unclear, however, and, for example, it could affect the permeability behaviour and stability of the granule membrane, as has been suggested for insulin secretion granules (Hellman, 1975), and histochemical studies at the ultrastructural level have demonstrated calcium deposits associated with some secretory granules (Herman *et al.*, 1973).

The results obtained with Triton and digitonin strengthen the argument that renin is present in

membrane-bound granules. The similar pattern of renin and acid phosphatase release in the presence of digitonin suggests a similarity in the cholesterol content of the renin-granular and lysosomal membranes, since the lytic action of digitonin is related to the cholesterol content of the membrane (Kirschbaum, 1976). Although ATP, cyclic AMP and cyclic GMP did not alter the release of renin from the partially purified granular fraction (Table 5), these results do not necessarily exclude a role for these substances in the mechanism of renin release, since the actions of these agents probably depend on other unknown factors and the appropriate intracellular ionic conditions. The results are, however, consistent with those obtained from perfused rat kidneys, where cyclic AMP and cyclic GMP did not alter significantly renin secretion (Peart *et al.*, 1975). Yamamoto *et al.* (1973) reported that cyclic AMP had no effect on renin release from dog kidney renin granules isolated by sucrose-density-gradient centrifugation.

However, in another study, in contrast with the present investigation, they found that ATP and calcium increased the renin release (Yamamoto *et al.*, 1974). Hong & Poisner (1976) have also demonstrated that ATP does not alter release from a particulate preparation isolated from rat kidney-cortex homogenates. Hong & Poisner (1976), as in the present study, also found a similar response to pH, osmotic factors and to a bivalent cation (Mg^{2+}). However, in contrast with the present investigation they found that renin release from their granule preparations increased with decreasing temperature when incubated in a KCl buffer. These discrepancies in results might reflect differences in incubation conditions and organelle composition of the particulate renin fractions employed.

The results obtained in this investigation suggest that renin in the kidney cortex is stored within membrane-bound granules whose physicochemical properties in response to a variety of conditions are similar to those of renal lysosomes and in certain respects also to those of other secretory granules.

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