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CHARACTERISTICS OF RENIN RELEASE FROM ISOLATED SUPERFUSED GLOMERULI *IN VITRO*

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SUMMARY

1. A method is described for studying renin release from superfused rat glomeruli following their rapid isolation by a magnetic iron-oxide technique.

2. Microscopically selected glomeruli were free of tubular components. Some possessed vascular pole protrusions of up to 20 μm, unrelated to renin content.

3. Renin content of 102 batches, each of 400 glomeruli, was $1{\cdot}34 \pm 0{\cdot}08 \times 10^{-4}$ Goldblatt hog units per 100 glomeruli ($\pm$ S.E. of mean). Different osmolarities (305, 355 and 400 m-osmole/l.), sodium concentrations (110 and 135 mM) and buffer compositions of the preparation solution did not alter this value. Renin content per glomerulus in intact kidney was 100-fold higher.

4. At 30° C the contained juxtaglomerular cells released renin at consistent but decreasing rates over 4–6 hr. Initial release rate in 110 mM sodium, 305 m-osmole/l. solutions was $0{\cdot}86 \pm 0{\cdot}068 \times 10^{-6}$ units per 100 glomeruli per 30 min ($\pm$ S.E. of mean, $n = 42$) or $0{\cdot}546 \pm 0{\cdot}043$ % of content per 30 min. In 135 mM sodium, 305 m-osmole/l. solutions, release was 2·4-fold higher ($P < 0{\cdot}001$) and remained elevated for at least 3 hr. When related to renin content per glomerulus resting release rate *in vitro* was higher by at most one order of magnitude than calculated *in vivo* values.

5. Release was augmented by gentle physical agitation of the glomeruli.

6. Release rate was inversely related to temperature. On reducing temperature from 30° C, release increased 2·6-fold at 20° C and 6·7-fold at 10° C ($P < 0{\cdot}001$, $n = 11$). The response was reversible.

7. 3 mM sodium cyanide plus 3 mM sodium iodoacetate caused a variable

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release of renin associated with depletion of content within 4 hr. The response was progressive and reached a peak after 60 min.

8. Sensitivity of renin release to temperature and metabolic blockade indicates that energy is required for retention of renin by the cell. This, together with the release observed with increased sodium concentration at constant osmolarity, suggests a dependence of renin release upon the mechanism controlling the volume of the juxtaglomerular cell or its organelles.

INTRODUCTION

The juxta position of the renin containing cells of the renal afferent arteriole both to the glomerulus and to the macula densa region of the distal nephron remains the most compelling evidence that renin secretion is linked in some manner with the autonomous nature of individual nephron function. The contribution made by morphologists to this concept was reviewed by Smith (1940) while pertinent subsequent works are those of Cook & Pickering (1959), Faarup (1968), Cook (1971) and Barajas (1972). Functional studies have as yet been unable to clarify this concept, and whether normal variations in the composition or volume of tubular fluid at the macula densa can influence renin secretion and whether such secretion acts in a close feed-back within the individual nephron are questions as challenging today as when first formulated by Goormaghtigh (1937).

McManus (1942) first suggested that micropuncture experiments 'of the type made by Richards might determine the condition in the distal tubule, whether chemical or hydrostatic which affects the flow through the glomerulus'. Recently evidence has been presented that contrived alterations in the compositions of distal tubular fluid can influence single nephron filtration rate and filtration pressure in the rat kidney (Thurau, Schnermann, Nagel, Horster & Wahl, 1967; Schnermann, Wright, Davis, Stackelberg & Grill, 1970; Schnermann, Persson & Ågerup, 1973) but that this might be mediated via renin secretion, and that such secretion could possibly also influence tubular function (Leyssac, 1965) as part of an autonomous nephron response remains speculative.

Whereas it has been convincingly demonstrated using kidneys subjected to extensive tubular damage (Blaine, Davis & Witty, 1970; for review see Davis, 1973) that renin secretion can respond to certain stimuli in the absence of a change in tubular fluid composition, such experiments cannot test directly the notion that renin secretion is normally subject to tubular influences and that such influences are controlling factors in individual nephron autonomy. In order to approach this problem a study of the characteristics of renin release from juxtaglomerular cells isolated free

from all surrounding influences seemed a worthwhile prelude to the ultimate study of rates of renin secretion by the single nephron *in vivo*.

Existing techniques such as kidney slices or kidneys subjected to extensive tubular damage were not satisfactory for these requirements since they necessarily leave the renin secreting cells within and towards the end of a contractile conduit. In addition, the cells in such preparations remain in intimate contact with the macula densa albeit without tubular flow. Whether the cells could be isolated in a viable state and whether a response in renin release might then have relevance to physiological function are questions examined in the present paper.

METHODS

Sprague–Dawley rats of either sex, weighing 200–300 g, were anaesthetized with sodium amytal (12·5 mg/100 g body wt. I.P.), the trachea cannulated and the left kidney exposed through a wide T-shaped mid-line abdominal and connecting left flank incision. A loose clamp was positioned around the aorta above the renal arteries and a polyethylene cannula was introduced into the aorta from above the bifurcation and advanced to the level of the renal arteries below the aortic clamp. The cannula was filled with isotonic saline containing heparin. Magnetic-iron oxide particles that had been prepared previously by the method of Cook & Pickering (1959) and suspended in 100 ml. distilled water were diluted 1 in 20 in the superfusion buffer (*vide infra*) containing 1 % bovine serum albumin and 1000 u. heparin in a total volume of 20 ml. This volume was injected over 5 min into the aortic cannula after closure of the suprarenal aortic clamp. The kidneys blanched during successful perfusions. Hydrostatic pressure in the perfusing system was continuously monitored and kept between 60 and 100 mmHg. The iron particles were prevented from settling by gradual rotation of the syringe. It was considered that suspension of the particles was facilitated by the presence of the 1 % albumin; higher concentrations lowered the pH of the buffer.

Following perfusion, the kidneys were removed and the cortices sliced thinly with a scalpel blade and the slices gently pressed through a 150 μm mesh stainless-steel sieve using the tip of the finger. The sieve was washed clean with the superfusion buffer and the brei was immediately passed through a length of glass tubing (4 mm internal bore) that lay between the poles of an electro-magnet, the specifications of which are given below. By adjusting the fluid flow rate and the field strength, thousands of iron-containing glomeruli were isolated within 15 min of the iron injection and with minimal contamination from larger tubular elements. The magnet was turned off, and the glomeruli flushed into a Petri-dish from whence they could be individually selected under binocular microscopy. All these procedures were conducted at room temperature (22–24° C). The method to this stage was similar in principle to that of Cook & Pickering (1959) and has been shown by Brendel & Meezan (1973*a*) to yield a 95 % pure preparation of glomeruli.

For the present purposes a continuous flow system (Fig. 1) was preferable to static incubation in order to more precisely document secretion with respect to time. Four hundred glomeruli, selected free of attached tubular elements and other fragments were withdrawn by careful mouth-suction into each of five polyethylene tubes (internal diameter 0·58 mm) that now lay between the poles of the magnet (Fig. 1). The selected glomeruli accumulated within the magnetic field where they

were subsequently superfused at rates of 280 μl./hr from one of a pair of constant infusion machines (Braun) each of which carried five 2·5 ml. polyethylene syringes. Each superfusion line containing the glomeruli was connected with two syringes, one on each infusion machine. This arrangement permitted changes from a control buffer to a test solution and back to control without disturbance to the glomeruli. Water was run at constant monitored temperatures between 10 and 36° C through a glass jacket that surrounded the superfusion lines. The magnetic field was maintained during all superfusion periods in order to prevent the glomeruli from moving. This was confirmed by direct observation of the glomeruli by binocular microscopy as they lay within the lines.

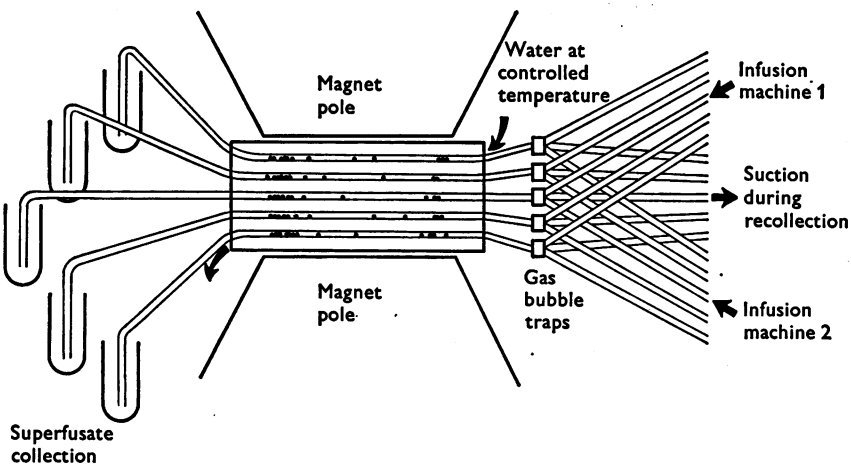

Fig. 1. A diagrammatic representation of the superfusion apparatus. The isolated glomeruli are shown as dots in five lines. They are held in space by a divergent magnetic field and superfused by one of two infusion machines. During superfusion the suction lines were closed.

Superfusate (140 μl.) from each line was collected during periods of 30 min into narrow tubes containing 2 μl. 25 % human serum albumin (HSA) as stabilizer (State Serum Laboratory, Copenhagen). Following collection the tube was stoppered and the sample stored frozen for later renin assay. Evaporative losses were negligible. At a superfusion rate of 140 μl./30 min the time required to flush the dead space of the system on changing from one infusion machine to the other was 10 min. Half of this time was lost in replacing the solution in a small air trap at the beginning of the superfusion lines (Fig. 1). The remaining 5 min was the time taken for the new front of solution to reach the collecting test tubes. Formation of air bubbles within the narrow tubes was avoided by bubbling the superfusion solutions with their appropriate gas mixtures at 37° C and then allowing the solution temperature to fall to a lower monitored level during superfusion.

At the end of each experiment the magnet was switched off and each line was flushed with 500 μl. of solution. In this manner the contained glomeruli were collected in small test tubes, stabilized with 2 μl. 25 % HSA and then frozen for later renin assay.

In most experiments the superfusion solution was a bicarbonate–Ringer pH 7·4,

containing 110 or 135 mM sodium bubbled with 5% CO_2 and 95% O_2. The composition of the 110 mM sodium solution in m-mole/l. was: NaCl 91·0; $NaHCO_3$ 17·5; KCl 7·0; $CaCl_2$ 2·0; $MgSO_4$ 1·2; NaH_2PO_4 1·2; glucose 11·0; sucrose 49·0. The calculated ideal osmolarity of this solution is 305 m-osmole/l. Direct estimations of osmolarity were not performed. Osmolarity was changed as indicated in results through manipulation of sodium and sucrose concentrations.

Comparison was made between this solution and phosphate–Ringer containing 108 or 133 mM sodium in which case the solution was bubbled with 100% O_2. The 108 mM sodium solution had a calculated osmolarity of 305 m-osmole/l. with the following composition in m-mole/l.: NaCl 105·4; KCl 7·0; $CaCl_2$ 2·0; $MgSO_4$ 1·2; NaH_2PO_4 0·32; Na_2HPO_4 2·13; glucose 11·0; sucrose 50·4; pH 7·4.

When using the CO_2-bicarbonate solution for preparation of glomeruli, care was taken to avoid loss of CO_2 from open fluid surfaces. During the re-collection of the selected glomeruli from the Petri-dish an atmosphere of 5% CO_2 and 95% O_2 was maintained over the solution. A check on the pH was made at all stages. In some experiments sodium cyanide and iodoacetate were dissolved in the buffers in concentrations stated in the text.

Renin concentration in the superfusate, glomeruli, whole kidneys and plasma was determined by radioimmunoassay of generated angiotensin I formed during incubation of superfusate with nephrectomized rat plasma as the source of substrate. The assay was a direct application of the 'trapping' method of Poulsen & Jørgensen (1974) designed originally to reduce interference from angiotensinase and to remove the need for pre-treatment of the reactants. Superfusate (25 μl.) were incubated for 2–3 hr at pH 7·4 with 25 μl. renin substrate providing a substrate concentration in the incubate of 2·0 μM expressed as total content of angiotensin I. All assays were performed in duplicate. Assays were performed in batches of sixty and included standard displacement curves for both angiotensin I and renin. In the absence of an accepted standard for rat renin, assays were made referable to the Goldblatt hog renin unit available as a standard preparation from the National Institute for Biological Standards and Control, Holly Hill, London. The possibility that preformed angiotensin I might be secreted together with renin (Finkielman & Nahmod 1969; Johnston, Mendelsohn, Hutchinson & Morris 1973) was tested but could not be confirmed, at least down to concentrations of 100 pg/25 μl. superfusate. In addition, the influence of different buffers and drugs upon the displacement curve was checked in each new situation, but no effect was discernible.

Renin content of whole kidneys was determined after dicing and sieving the kidneys as in the initial steps for isolation of the glomeruli, followed by the addition of 100 ml. distilled water and then freezing and thawing four times. Renin content of glomeruli whether single or in batches was also estimated after freezing and thawing four times. In preliminary studies no alteration was found in renin content between a second, third, fourth and a fifth thaw. In addition a comparison was made of renin content in standard aliquots of isolated glomeruli after extraction in 10% ether, after freezing and thawing four times, and after acidification by dialysis to pH 2·8. The acidification and the freezing-thawing sequence yielded the same values. Renin failed to survive the ether treatment. Ultrasonication of glomeruli was also attempted but recovery of small volumes was not reproducible.

An electromagnet with special conical poletips was constructed for the present requirements (Fig. 2). The poletips were designed to provide a strong field with maximum divergence by placing a knife-edge poletip opposite an arched poletip (r = 5 mm). The material used was soft iron. The magnetic induction at the knife-edge was 1·4 Wb/m^2. This induction required a magnetomotive force of 4500 ampere-turns between the two areas supporting the polehead. The magnetic flux supplied to these areas was 0·0030 Wb. The leakage flux between the poles was high.

The driving magnetomotive force was supplied by a U-shaped electromagnet with two coils operating at low power (22 W). Minimal heating of the poletips occurred because the magnet core (area 50 cm^2) was to a large extent thermally isolated from the magnet coils. In addition the yoke of the core was furnished with cooling fins. Thus the steady-state temperature rise of the core and the poletips (5° C per hour) was about half the temperature rise of the coils. Natural air cooling reduced the temperature rise to around 2° C per hour at the poletips.

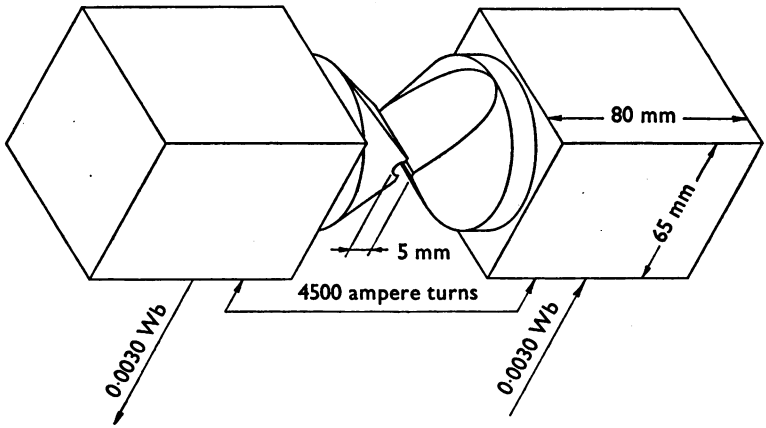

Fig. 2. Specifications of the polehead and poletips.

Each of the two coils had 6700 turns of 0·8 mm diameter Cu-wire, 92 Ω, and was excited by 0·34 A at 32 V. The d.c.-voltage for the coils was supplied by a single-phase, bridge coupled rectifier fed by a variable auto transformer. The weight of the magnet was 57 kg, and the polehead 6·5 kg.

RESULTS

I. *Renin in isolated glomeruli and whole kidneys*

Table 1 shows the content of renin in batches of 400 glomeruli prepared in the various superfusion solutions used in these experiments. Content did not vary significantly with respect to sodium concentration, osmolarity or buffer composition.

The mean renin content in all 102 batches of 400 glomeruli was $1{\cdot}34 \pm 0{\cdot}08 \times 10^{-4}$ units per 100 glomeruli (± S.E. of mean). This content was only one-hundredth that associated with glomeruli *in situ* in whole kidney, a conclusion reached after estimating renin content in 12 kidneys ($3{\cdot}6 \pm 0{\cdot}144$ units per kidney) and assuming there to be 30,000 glomeruli per kidney (Arataki, 1925; Vimptrup, 1928; Rytand, 1938; Baines, de Rouffignac & Deiss, 1969). The amount of renin was calculated to be $1{\cdot}2 \pm 0{\cdot}048 \times 10^{-2}$ units per 100 glomeruli (± S.E. of mean). Whether kidneys were injected with iron oxide particles or not, did not influence

this value ($1{\cdot}16 \pm 0{\cdot}100 \times 10^{-2}$ and $1{\cdot}25 \pm 0{\cdot}124 \times 10^{-2}$ units per 100 glomeruli respectively). Renin released into the cell-free supernatant during preparation of the kidney brei and magnetic separation of the glomeruli was $0{\cdot}40 \pm 0{\cdot}072$ units per kidney ($\pm$ S.E. of mean, $n = 10$). Thus, approximately three units remained in the non-glomerular particulate fraction of each sieved kidney, presumably associated with fragments of afferent arteriole.

TABLE 1. Renin content in batches of glomeruli prepared in different solutions*

Buffer	Sodium (mM)	Osmolarity (m-osmole/l.)	No. of batches of 400 glomeruli	Renin content ± S.E. of mean (units × 10^{-4}/ 100 glomeruli†)
CO_2-bicarb.	110	305	29	$1{\cdot}65 \pm 0{\cdot}22$
CO_2-bicarb.	110	355	37	$1{\cdot}12 \pm 0{\cdot}089$
CO_2-bicarb.	110	400	10	$1{\cdot}77 \pm 0{\cdot}24$
CO_2-bicarb.	135	305	11	$1{\cdot}27 \pm 0{\cdot}10$
Phosphate	110	305	10	$1{\cdot}50 \pm 0{\cdot}10$
Phosphate	110	400	5	$1{\cdot}78 \pm 0{\cdot}21$

* Osmolarity adjustment was through changes in sucrose concentration.
† No significant differences at the 5 % level.

II. *Histology*

Under binocular microscopy the majority of glomeruli appeared as a ball of capillaries without attached tissue. A variable number, less than 10 %, displayed protrusions which were either short lengths of proximal tubule or more frequently up to 20 μm of vascular pole tissue. In order to establish whether these latter glomeruli possessed most of the renin a relationship between renin content and possession of a pole was sought in twenty-five selected glomeruli. Renin content varied widely from barely detectable levels of 10^{-7} up to 4×10^{-5} units per glomerulus and values bore no relationship to the possession of a protrusion at the vascular pole. Sections of the glomeruli stained with haematoxylin and eosin or periodic-acid-Schiff and examined under light microscopy failed to reveal any tubular elements in the vascular pole area. There were however short arteriolar protrusions from a considerable number of glomeruli while only occasional ones retained the epithelium surrounding Bowman's space.

III. *Renin release from isolated superfused glomeruli*

Whether the glomeruli were prepared and superfused in phosphate or bicarbonate-containing solutions did not influence resting renin release rate nor was there any discernible effect when changing the superfusate from one buffer solution to the other at constant sodium concentration and osmolarity.

Fig. 3 shows the results from thirteen preparations each of 400 glomeruli superfused for a period of 210 min at 28–30° C. Nine of these preparations were superfused with a solution containing 135 mM sodium at 305 m-osmole/l., and four with a solution of 110 mM sodium, also at 305 m-osmole/l. In most cases there was a steady decrease in renin release with time. For statistical testing, the time interval 30–60 min was chosen since the glomeruli had by then been exposed to 30 min of stable conditions

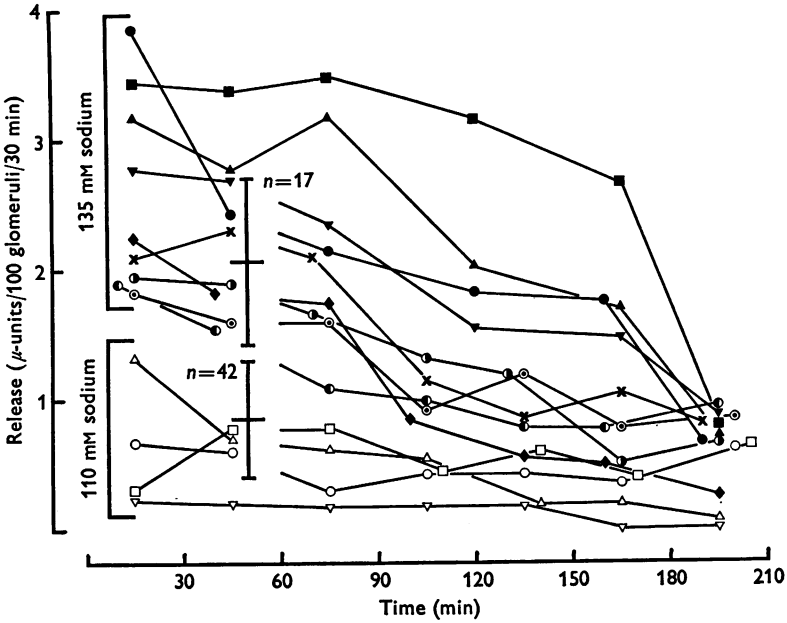

Fig. 3. Pattern of renin release from glomeruli during prolonged periods of superfusion with solutions containing either 135 mM sodium (above) or 110 mM sodium (below) each of 305 m-osmole/l. The ranges of release during the period 30–60 min using these two solutions are shown as means ± 1 S.D. for all experiments in the series ($P < 0{\cdot}001$). Following this period superfusate composition was altered in those experiments which are included in the calculation of mean release rate but are not plotted in the Figure.

while following this, many preparations (not plotted in Fig. 3) were subjected to changing conditions. Table 2 shows release rate during this period for all three superfusion solutions used in these experiments, and also shows the amount of renin released during this 30 min period as a percentage of the total amount initially present in the 400 glomeruli. Compared with release rate in 110 mM sodium 305 m-osmole/l. superfusate, a rise in sodium concentration at constant osmolarity increased release, while a rise in osmolarity at constant sodium concentration had no effect.

Fig. 4 shows that on reducing the rate of superfusion by half, the concentration of renin in superfusate rose to an extent that was consistent with unaltered release rate. Thus, the expected concentration in the absence of a flow rate change (mean of values immediately before and after flow

TABLE 2. Renin release during the period 30–60 min with varying sodium and osmolar* concentrations of superfusate at 29° C

Sodium (mM)	Osmolarity (m-osmole/l.)	No. of experiments	Release ± S.E. of mean (units × 10^{-6}/100 glomeruli per 30 min)	% of content released ± S.E. of mean
135	305	17	2·062 ± 0·156	1·62 ± 0·126
110	305	42	0·860 ± 0·068 ($P < 0·001$)	0·546 ± 0·043 ($P < 0·001$)
110	355	28	0·686 ± 0·068 ($P < 0·001$)	0·61 ± 0·063 ($P < 0·001$)

* Osmolarity adjustment was through changes in sucrose concentration. Probability levels were calculated using Student's *t* test to analyse the difference between the upper and each of the lower two solutions.

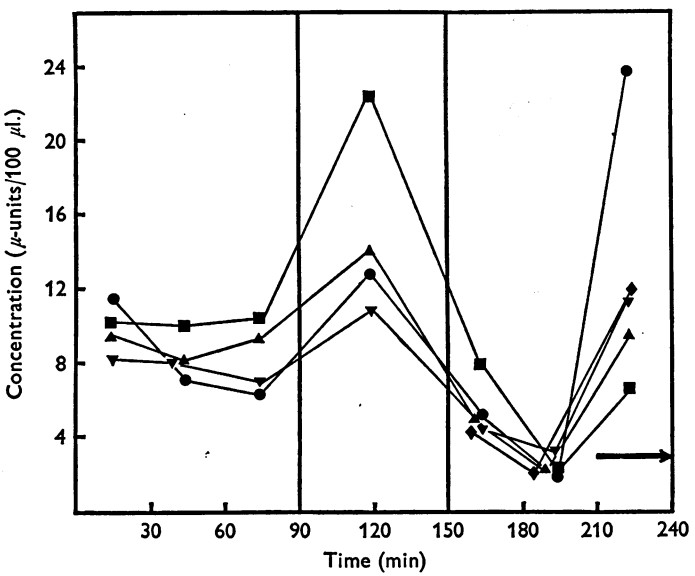

Fig. 4. The effect of reducing the rate of superfusion from 140 to 70 μl./30 min (central section) on the concentration of renin in the superfusate. The observed mean increase was 2·13-fold above the average of the pre- and post-control levels. The effect of physical agitation during the period 210–240 min is also shown. Superfusion solution contained 135 mM sodium and 305 m-osmole/l. Superfusion was performed at 29° C.

reduction) was $7{\cdot}0 \times 10^{-6}$ units per 100 μl. and the observed mean concentration at reduced flow rate was 2·13 times higher. These four perfusion lines are the top four experiments in Fig. 3 (symbols ●, ■, ▲, ▼) where it can be seen that the rate of renin release is not affected by the change in rate of superfusion occurring at 120 min. Fig. 4 also shows the increase in renin concentration in the superfusate resulting from physical movements of the glomeruli. Agitation was achieved by gently tapping the superfusion lines approximately once every 10 sec during the 30 min period.

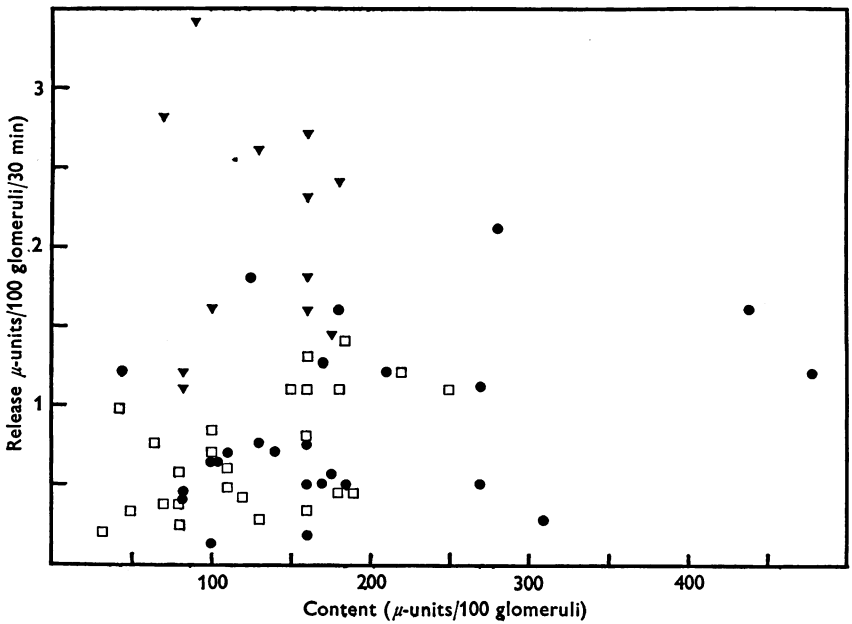

Fig. 5. Scattergram of renin release rate in various superfusion solutions during the period 30–60 min (Table 1) plotted against renin content of the glomeruli at the commencement of the experiment. ▲ 135 mM sodium, 305 m-osmole/l.; ● 110 mM sodium, 305 m-osmole/l.; □ 110 mM sodium, 355 m-osmole/l.

The force exerted on the glomeruli could not be quantitated but under binocular microscopy the glomeruli were seen to oscillate through about 0·1 mm. Expressed as percentage of content present prior to agitation, the procedure released at most only 5% of the contained renin. No glomeruli were displaced from the magnet area by this procedure, nor were any fragments seen to break away. It was considered that this procedure subjected the glomeruli to less distortion than during the initial preparation and collection period. Whereas both content (Table 1) and release (Table 2) of renin varied considerably between different experi-

ments there was no apparent relationship between content and release within individual experiments. Fig. 5 is the scattergram of these data for various compositions of superfusate.

IV. *Comparison between in vivo and in vitro release rates*

The rate of renin release *in vivo* was derived in two ways. Arterial plasma renin concentration was measured by the present method in twenty-nine rats that had been anaesthetized with sodium amytal. The mean value was $2{\cdot}67 \pm 0{\cdot}27 \times 10^{-4}$ units/ml. ($\pm$ S.E.). In 250 g anaesthetized rats renal venous renin level is 1·4 times higher than the arterial level and renal plasma flow is 3 ml./min per kidney (Churchill, 1973) making renin production per kidney $9{\cdot}6 \times 10^{-3}$ units/30 min. Assuming there to be 30,000 glomeruli per kidney, renin release per 100 glomeruli was 32×10^{-6} units/30 min. Expressed in relation to renin content, release *in vivo* was 0·27 % of content/30 min.

Alternatively, assuming the half-life of renin in rat plasma to be 16 min and the renin distribution space to be approximately 36·5 ml. in 250 g rats (Peters-Haefeli, 1971), at the constant arterial level of $2{\cdot}67 \times 10^{-4}$ units/ml., production rate from two kidneys would be $9{\cdot}1 \times 10^{-3}$ units/30 min. Release per 100 glomeruli would therefore be $15{\cdot}2 \times 10^{-6}$ units/30 min or 0·13 % of content/30 min.

By either calculation, when related to renin content per glomerulus release rate from isolated glomeruli was higher (Table 2) but was within an order of magnitude of the estimated *in vivo* values.

V. *Effect of temperature on renin release*

Fig. 6 shows the effect of reducing temperature of the superfusate by means of the external water jacket in eleven experiments. Temperature was reduced in two steps and in most experiments two 30 min periods of superfusion were performed during the first step at 20° C. Solutions with various sodium, osmolar and buffer compositions were employed as indicated in the legend. Release rate increased with fall in temperature in all cases. The increase above control was 2·6-fold in the first period at 20° C ($P < 0{\cdot}001$, $n = 11$ Student's t test on paired data) and 6·7-fold at 10° C ($P < 0{\cdot}001$, $n = 11$). Release during the first 30 min period at 20° C was higher than during the second ($0{\cdot}002 < P < 0{\cdot}01$, $n = 6$). The cumulated total of renin released during these procedures was less than 5 % of the renin present in the glomeruli at the beginning of the experiment. The effect of temperature reduction within this range was the same whether tested early or late in the experimental protocol.

On changing superfusion temperature within the range of 30–36° C in

either upward or downward directions, no consistent change in release rate was detected in twelve separate experiments. These latter observations were, however, made late in the experimental protocol and the consequent low secretion rate could have masked a small influence of temperature within this range.

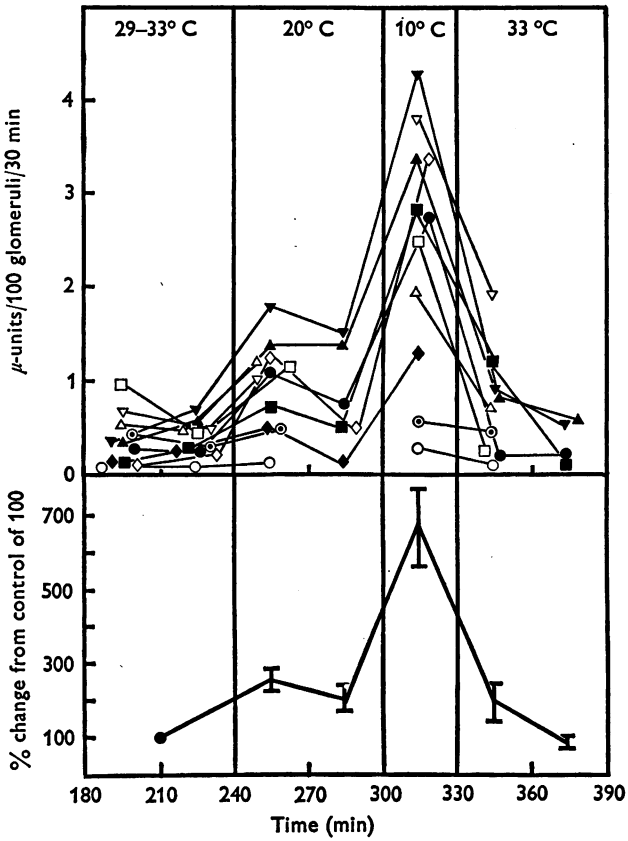

Fig. 6. Effect of temperature upon rate of renin release from isolated glomeruli. Above: individual values for all experiments. Below: mean percent change from control expressed as 100 (± S.E.). Closed symbols denote the use of phosphate buffer and open symbols of CO_2-bicarbonate. Concentrations of sodium (mM) and osmolarity (m-osmole/l.) were respectively ● ■ ○ ▲ ▼ 110, 305; ◆ ◇ 135, 305; □ 110, 280; ▽ ⊙ 135, 355; △ 110, 255.

VI. *Effect of sodium cyanide and iodoacetate on release and content*

Fig. 7 shows the results of nine experiments in which the glomeruli were exposed to a combination of 3 mM sodium cyanide and 3 mM iodoacetate. The degree of response varied widely. In six there was a highly

significant release persisting for over 2 hr; in a seventh the response was slight, whilst in two the gradual decline in resting release typical of control preparations was not altered. The three experiments in which the response was small or absent were all performed with the CO_2-bicarbonate buffer, while the superfusate for the others contained a phosphate buffer. The possibility that the nature of the buffer is related to the size of the response has not as yet been further investigated.

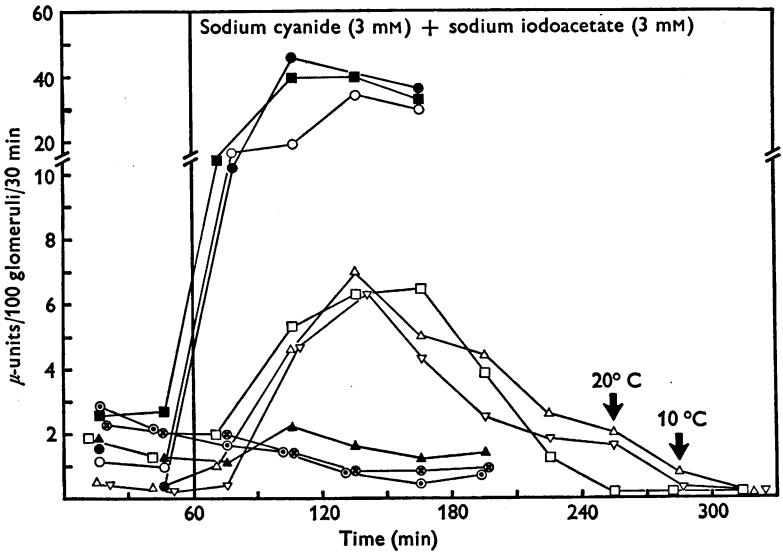

Fig. 7. Effect of metabolic blockade introduced at the vertical line upon renin release from isolated glomeruli superfused at 30° C. Sodium (mM) and osmolarity (m-osmole/l.) were respectively 135 and 305 for filled symbols and 110, 305 for open symbols. The three experiments showing little or no response were performed with a CO_2-bicarbonate buffer, others with phosphate. Temperature was reduced from 30 to 20° C and then 10° C during the 30 min periods indicated by the arrows.

One difference between the responding and non-responding glomeruli was found in renin content at the termination of the experiment. In the six in which a large response occurred the final renin content was greatly reduced at $0{\cdot}237 \pm 0{\cdot}121 \times 10^{-4}$ (S.E.) units/100 glomeruli which should be compared with the content in nine control lines in these same experiments ($1{\cdot}34 \pm 0{\cdot}118 \times 10^{-4}$ units/100 glomeruli, $P < 0{\cdot}001$). In the three non-responding lines the mean content was $1{\cdot}0 \times 10^{-4}$ units/100 glomeruli (actual values of 1·2, 1·2 and 0·6). Thus, only when release occurred with metabolic blockade was there an associated depletion of renin.

The onset of release varied with the magnitude of the response and in all cases reached a peak 30–90 min after introducing the metabolic blockers (Fig. 7). Importantly, release during the first 30 min period with blockers was never as high as during the second, and in four experiments no effect at all could be discerned during the first period. In three of the experiments, reduction in temperature firstly from 30 to 20° C and then to 10° C, was not associated with the expected augmentation of release (Fig. 7). Lower concentrations of metabolic blockers (0·3 mM) were studied in only two experiments and in neither was release provoked, nor was there increased release in two experiments with 3 mM sodium cyanide alone nor in one experiment with 3 mM iodoacetate alone. All of these latter experiments were performed with the phosphate buffer.

DISCUSSION

The technique was developed to further elucidate the normal function of the juxtaglomerular (J–G) apparatus. The main advantage over previous systems for studying renin release *in vitro* was the possibility of monitoring release over many hours from the same preparation in the absence of tubular, vascular and neural influences. However, unless it can be accepted that the J–G cells as presently prepared remain viable through the preparative and experimental periods and are therefore capable of yielding physiological information, the technique, as such, is valueless.

Glomeruli prepared in identical fashion remain metabolically active, consume oxygen, incorporate amino acids into particulate protein and nucleotides into nucleic acid (Brendel & Meezan, 1973*a*, *b*). In addition metabolic activity is unaffected by the presence of iron (Brendel & Meezan, 1973*a*, *b*). These results, published during the course of the present work, establish that at least some cells are viable. In our experiments using renin assay as a probe of J–G cell function, it was shown that a small fraction of the renin content is released at consistent rates over many hours; that release is sensitive to sodium concentration at constant osmolarity; that release is inversely related to temperature and is augmented by metabolic blockers. Furthermore, in the simulated *in vivo* electrolyte environment of the cell, renin was released at rates only slightly higher than normal physiological secretion rates when related to renin content. These criteria establish with reasonable certainty that the cells are viable and should be capable of functioning normally.

Glomeruli as presently prepared possess only one-hundredth the renin associated with each glomerulus *in vivo*. Severance of the afferent arteriole within 50 μm of the glomerular corpuscle is the likely reason for this because Faarup (1968) has shown in cats that some renin-containing cells

occur several hundred microns from the glomerulus. However, the possibility that the few J–G cells contained in the corpuscle have lost a significant amount of their renin content during preparation cannot be excluded. Certainly large quantities of renin appeared in the supernatant during preparation of the kidney brei but this is presumed to have come from damaged cells. The fact that once isolated, the glomeruli release only a few percentage of their renin content in response to considerable physical agitation, leads to the conclusion that whereas some renin must leave the cells during preparation the amount could not have caused severe depletion.

Initially, it seemed likely that by comparing the final renin concentration in pools of glomeruli that had been stimulated to release renin with untreated controls, it would be possible to calculate whether synthesis had replaced the lost renin. In the event, the amount of renin released by physiological stimuli was such a small fraction of the total that it lay within the error of replicate estimation of content. Nor was there any indication of the type of response reported by Thurau, Dahlheim, Grüner, Mason & Granger (1972) of rapid increases in glomerular renin content nor any evidence for secretion of preformed angiotensin I (Finkielmann & Nahmod, 1969; Johnston *et al.* 1973).

On comparing the content of renin in glomerular corpuscles with that in the carefully microdissected glomerulus and its arteriole (Thurau *et al.* 1972) it is apparent that four fully dissected J–G apparatuses possess the same quantity of renin as 400 isolated glomerular corpuscles. In spite of the fact that only 0·2–2·0 % of this quantity was released each 30 min into 140 μl. superfusate the released amount was above the threshold of the assay and it was therefore not necessary to develop other preparative methods that would include the entire afferent arteriole. However, because Cook & Pickering (1959) found that glomeruli with arteriolar protrusions do contain more renin than those without, a brief study was undertaken to see whether such selection would provide some advantage. This proved not to be the case. Individual glomeruli varied greatly in their renin content but this was not obviously related to the possession of a protrusion. Apart from the added technical difficulty, selecting glomeruli with long afferent arterioles has the disadvantage of including more smooth muscle and thereby increases the possibility that the release could occur indirectly through distortion effects from surrounding contractile elements or from releasing factors, such as noradrenaline present in the muscular part of the vessel.

A further aspect of the renin content of glomeruli as presently prepared was the lack of a relationship between content and resting release rate under standard conditions. The source of variation in content (up to 5-fold in 102 batches each of 400 glomeruli) was only partly due to assay

variation since duplicate measurements performed on all batches varied by at most 30%. More important seems to be the chance inclusion of glomeruli with high renin content. Independence of release *in vitro* from renin content has also been noted in renal tissue slices by de Vito, Gordon, Cabrera & Fasciolo (1970). Variables that correlated more closely with release rate than did content, were the length of time of superfusion, the superfusion temperature and the superfusate composition. The decline in release rate with time could neither be accounted for by renin depletion nor cell death. Indeed, procedures directed to interfere with cell metabolism such as temperature reduction and metabolic blockade had just the opposite effect.

Reversible increase in renin release with moderate temperature reduction has apparently not been previously reported, although improved release from tissue slices at 25° C as compared with 37° C has been noted by Yamamoto, Tanaka, Horiuchi & Ueda (1967) and Weinberger & Rosner (1972). Presumably on the assumption that the reverse would be the case, many workers have used low temperatures during the preparation of kidney slices and isolated glomeruli for renin studies (Forgacs & Gaal, 1971; Ochs, Lamberts, Saleh & Heintz, 1973; Veyrat & Rosset, 1972; Michelakis, 1971; Braverman, Freeman & Rostorfer, 1971). In our experiments a temperature reduction of 10° from 30 to 20° C increased renin release 2·6-fold in the first collection and 2·0-fold in the second, while a 10°-reduction from 20 to 10° C increased release 2·8-fold. Since metabolic blockade similarly caused renin release to increase, the data strongly suggest that active, enzyme-dependent processes are required for the retention of renin by the J–G cell. Such a conclusion does not deny that the process of renin ejection from the cell either in these experiments or differently provoked might involve active secretory processes. Active processes causing renin release have been invoked to explain results with tissue slices (Jong, 1969; Božović & Efendić, 1969; de Vito *et al.* 1970) but further work is required to establish this point.

Whereas the response to temperature reduction was consistent and reversible, the effect of metabolic blockers was variable. Both cyanide and iodoacetate were required in doses that are widely accepted to reduce energy supply to a minimum. The fact that this treatment did not cause release in all preparations and that the drugs separately or in lower doses were without effect belies the sensitivity observed during reduced temperature. Nevertheless, the results do indicate that the retention of renin in the cell is an energy requiring phenomenon.

The simplest interpretation of the results which also accords with the finding that secretion rates are higher in high sodium solutions at a constant osmolarity of 305 m-osmole/l. is that renin release bears a direct

and sensitive relationship to the volume of the J–G cell or some contained organelle. The fact that release rate falls gradually with time even though content is not detectably reduced, might then reflect recovery of the system regulating cell volume during the course of the superfusion experiment. These considerations lead to the possibility that one physiological mechanism controlling renin secretion is change in J–G cell volume possibly through an effect on membrane tension. This proposal has been tested in an extension of the present study where on changing osmolarity through sucrose concentration it was revealed that, with respect to renin release, J–G cells are sensitive osmometers with renin release doubled by a 7% fall, and halved by a 10% rise, in superfusate osmolarity (S. L. Skinner, O. Frederiksen & P. P. Leyssac, unpublished data). Apparently against this conclusion is the present observation that high superfusate osmolarity (355 m-osmole/l., Table 2) is not associated with suppression of renin release. However, the low level of release in the preparations used for comparison (110 mM sodium, 305 m-osmole/l., Table 2) and the time course of the changes in release following changes in osmolarity apparently precluded a significant effect in the present experiments; suppression of renin release with increase in sucrose concentration is seen more clearly immediately following acute increases in osmolarity (S. L. Skinner, O. Frederiksen & P. P. Leyssac, unpublished observations).

A long and often conflicting literature relates to this consideration and also to the associated possibility that glomerular filtration is controlled through swelling and/or release of renin from the J–G cell (Smith, 1940; Guyton, Langston & Navar, 1964; Vander, 1967; Yamamoto, Hasegawa, Tanaka & Ueda, 1968; Sokabe, Ogawa, Oguri & Nishimura, 1969; Forgacs & Gaal, 1971; Sokabe, Nishimura, Kawabe, Tenmoku & Arai, 1972; Weinberger & Rosner, 1972; Schnermann, Wright, Davis, Stackelberg & Grill, 1970; Young & Rostorfer, 1973). Most of these works are however relevant to the composition of fluid at the macula densa and not at the J–G cell itself. That J–G cell volume might be a physiological regulator of renin secretion in the manner proposed here has not apparently been advanced previously. In the intact animal, results have been obtained that could be taken as support or denial of this notion. Thus, increased renin secretion would be anticipated in situations where water enters cells, for instance during hyponatraemia. Such is apparent in the experiments of Yamamoto *et al.* (1968), Sokabe *et al.* (1972) and Blair-West, Brook & Simpson (1972) but is denied by the findings of Newsome & Bartter (1968) and Gordon & Pawsey (1971) where however changes in renal neurogenic tone through concomitant extracellular volume expansion could have reduced the renin releasing effect of hydration. Also apparently contrary to the suggestion is the finding of Young & Rostorfer (1973) that renin is

released in response to acute elevation in renal artery osmolarity using either hypertonic sodium, dextrose or urea. These latter authors explain this effect as the consequence of J–G cell shrinkage but for dextrose and urea the opposite seems possible because plasma sodium concentration must have been considerably reduced during the rapid injection of these permeant solutes and after a short time the result would be a movement of water into cells. Shrinkage of the cell could only be assumed with hypertonic sodium injections.

Different experimental designs are now required to test the present proposition *in vivo* but it is of particular interest that those J–G cells lying at the polkissen, within a few microns (Barajas, 1972) of the only hypotonic region of the body, as well as those lying at a distance under tension in the arterial wall are both subject to special local factors that could continuously challenge the volume of these cells and their intracellular organelles.

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