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THE ROLE OF CALCIUM IN RENIN SECRETION FROM THE ISOLATED PERFUSED CAT KIDNEY

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SUMMARY

1. Isolated cat kidneys were perfused *in situ* with Locke solution and renin release in response to isoprenaline was studied.

2. Perfusion with isoprenaline produced a concentration-dependent enhancement of renin secretion. Increasing the concentration of stimulant also prolonged the duration of the secretory response.

3. After a 10 min exposure to isoprenaline (0·3 μM), there was a rapid facilitation of renin release which diminished after 10–30 min, followed by a second transient increase which declined over the next 40–60 min. Cycloheximide did not prevent augmented release when added together with the isoprenaline but did produce a reversible inhibition of the late phase when added 10 min after the isoprenaline.

4. Omission of calcium from the perfusion medium failed to depress the renin release induced by isoprenaline, glucagon, or furosemide. However, during prolonged calcium deprivation, the cycloheximide-sensitive phase of isoprenaline-evoked release was depressed.

5. The calcium antagonist D-600 failed to block the early phase of isoprenaline-induced renin secretion but inhibited the late phase of secretion.

6. Calcium alone elicited an explosive discharge of renin when added after a prolonged period of calcium-free perfusion.

7. These results support the view that extracellular calcium does not play an essential role in the mechanism of renin secretion from the renal juxtaglomerular cells, but that an increased influx of this cation is needed for synthesis and/or mobilization of the enzyme. It is tentatively proposed that the release of calcium from intracellular storage sites may be the signal which triggers renin secretion.

INTRODUCTION

A vast amount of literature has accumulated regarding the factors controlling renin secretion by the juxtaglomerular cells of the kidney. In addition to the actions of intrarenal baroreceptors and catecholamines released from renal sympathetic nerves or from the adrenal medulla (Davis & Freeman, 1976), there is evidence that renin secretion is regulated by the effect of sodium concentration in the macula densa in the distal tubule of the nephron (Thurau, Schnermann, Nagel, Horster & Wohl, 1967; Vander, 1967). Other extracellular cations also appear to influence renin secretion (Davis & Freeman, 1976). Thus potassium, in contrast with its action in other secretory systems, appears to inhibit rather than facilitate renin release (Vander, 1970); and in the intact animal, infusion of calcium also appears to inhibit renin secretion, apparently by increasing the amount of filtered sodium presented to the renal tubule (Kotchen, Maull, Luke, Rees & Flamenbaum, 1974).

The well-established role of calcium as a mediator of the secretory process (Rubin, 1974) makes this cation a potentially important factor as a modulator of renin release through some direct action on the juxtaglomerular cell; this postulate has engendered support from a limited number of *in vitro* studies. Spontaneous renin secretion by canine kidney slices can be inhibited by calcium deprivation (Morimoto, Yamamoto, Horiuchi, Tanaka & Ueda, 1970; Michelakis, 1971). However, more compelling evidence favouring a direct role for calcium in renin release was the recent finding that calcium can directly activate renin secretion (Iwao, Abe & Yamamoto, 1974; Chen & Poisner, 1976).

The present investigation was undertaken to assess in a more comprehensive manner the direct role of calcium in the molecular mechanisms governing renin secretion. The preparation chosen for these experiments was the isolated kidney of the cat perfused *in situ* with Locke solution, which does not have the complicating neural and humoral influences commonly present *in vivo*; it also has the decided advantage of being a whole-organ preparation, thereby facilitating an accurate analysis of the kinetics of renin secretion which is not feasible in *in vitro* systems such as slices and isolated cortical cell preparations.

A preliminary account of some of these experiments has previously been reported (Lester & Rubin, 1976).

METHODS

Kidney perfusion. Under intraperitoneal pentobarbitone anaesthesia, cats (2–3 kg) were artificially ventilated and classical evisceration carried out. Adrenal glands, extra-renal vessels and the ureters were ligated. The left kidney was perfused

in situ at constant pressure by means of a cannula placed in the abdominal aorta according to a modification of the method for adrenal perfusion (Douglas & Rubin, 1961). Collection of the renal perfusate was carried out via a cannula placed in the renal vein. The perfusion medium was normal Locke solution of the following composition (mM): NaCl, 154; KCl, 5·6; $CaCl_2$, 2·0; $MgCl_2$, 0·5; $NaHCO_3$, 12; dextrose, 10. A mixture of amino acids was used as a source of substrate, and dextran (3%) was added to all solutions to maintain oncotic pressure and thereby reduce oedema. The perfusion medium was equilibrated with 95% O_2 and 5% CO_2 and had a pH of 7. In experiments in which the 2·0 mM calcium chloride normally present in Locke solution was omitted, ethyleneglycol-bis-(β-amino ethyl ether)-N,N′-tetra-acetic acid (EGTA) (0·2 mM) was added to chelate any residual calcium. Flow rate varied from preparation to preparation, ranging from 3 to 10 ml./min; but during the course of a given experiment, the constant pressure generally maintained a relatively constant rate which was not markedly altered by varying the ionic constituents. Drug additions, generally, had inconsequential effects on the perfusion rate; however, noradrenaline perfusion produced severe vasoconstriction, so isoprenaline was chosen as the primary stimulus for renin release. In certain experiments kidneys were perfused with mannitol (3 mM) for 60 min before ligation of the ureter. Since mannitol is filtered and not reabsorbed , its presence in the tubular lumen would severely reduce proximal tubular sodium and water reabsorption, thereby minimizing the possibility of replacement filtration. Experiments carried out on such preparations yielded results comparable to those obtained from preparations not perfused with mannitol, so effects on renin secretion were not deemed secondary to alterations in renal function.

Renin determinations. Perfusate samples collected over 10 min intervals from the renal vein were converted to angiotensin I and assayed for renin activity by a previously published radioimmunoassay technique (Haber, Koerner, Page, Kliman & Purnode, 1969). Aliquots of perfusate (50 λ) were incubated for 1 hr with renin substrate obtained from the plasma of anaesthetized cats whose renal vessels had been ligated for 5 hr before extraction of blood. Data were expressed either as μg renin released/min, or as a percentage of the output obtained during exposure to isoprenaline. Antibodies to angiotensin I were developed in albino rabbits according to the method of Caldwell, Burstein, Brock & Speroff (1971). After 6 months a 1:10,000 dilution of antiserum bound 56% of the homologous antigen. Although the antibody showed equal cross-reactivity with angiotensin I and angiotensin II in standard curve assays, converting enzyme inhibitors were added to incubation mixtures to block any angiotensin II formation, and the perfusion medium contained no interfering substances when assays were carried out on unconverted samples. Moreover, assays carried out using a commercial antibody with less than 0·1% cross-reactivity rendered values similar to those obtained with the less-specific antibody.

Substances used. Glucagon, cycloheximide and DL-isoproterenol were obtained from Sigma Chemical Company. A mixture of amino acids plus glutamine was obtained from Grand Island Biological Company. D-600 (as the hydrochloride salt), DL-propranolol, and furosemide were generously supplied by Knoll AG, Ayerst Laboratories and Hoechst-Roussel Pharmaceuticals, respectively. [^{125}I] angiotensin I and angiotensin I antisera were obtained from New England Nuclear. Stock solutions of all reagents were freshly prepared and diluted with Locke solution to desired concentrations.

RESULTS

The dynamics of isoprenaline-induced renin release

Kidneys were always perfused with normal Locke solution for 60 min before beginning collection of samples in order to wash out residual blood and to reduce secretory activity stimulated during preparation of the kidneys for perfusion. Perfusion with DL-isoprenaline for 10 min produced dose-related increases in renin release (Fig. 1). Isoprenaline concentrations as low as 0·3 nM were capable of enhancing secretion. As the concentration of stimulant was increased, the secretory response was prolonged so that

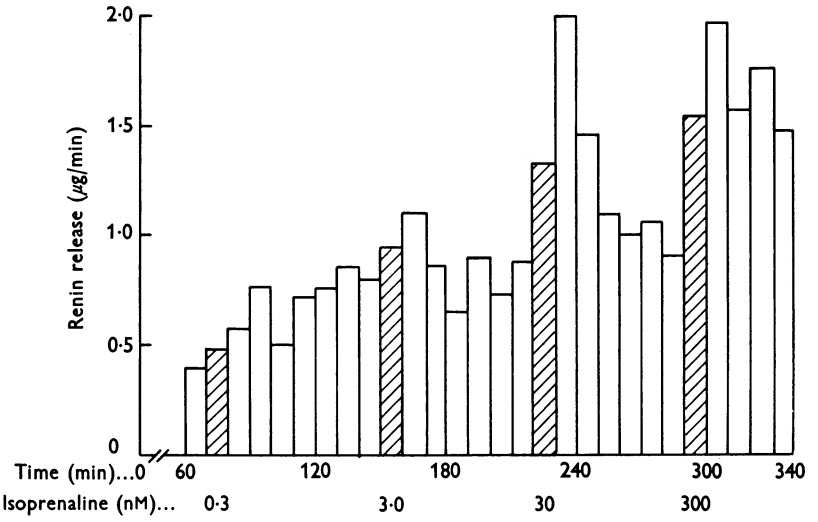

Fig. 1. Renin release during exposure to graded isoprenaline concentrations. A left kidney was perfused with Locke solution and varying concentrations of isoprenaline were added for 10 min every 70 or 80 min. The striped and open vertical columns represent renin outputs obtained during 10 min collection periods in the presence and absence of isoprenaline, respectively.

isoprenaline evoked maximum rates of renin release 10–40 min after exposure to the secretagogue (Figs. 1, 2*A*). The fall in the secretory rate following the rise after a 10 min infusion of the higher isoprenaline concentrations was in turn followed by a secondary rise, thus giving the response a typical biphasic appearance (Fig. 2*A*). Although the time of onset of the second phase varied from preparation to preparation, it generally occurred 10–30 min after cessation of the isoprenaline infusion.

The infusion of DL-propranolol prior to and during isoprenaline infusion markedly depressed the rise in renin release (Fig. 2*A*); however, if the

β-adrenergic blocking agent was added to the perfusion medium after exposure to isoprenaline, secretion was not noticeably affected.

Effects of cycloheximide. Since the biphasic nature of renin release might

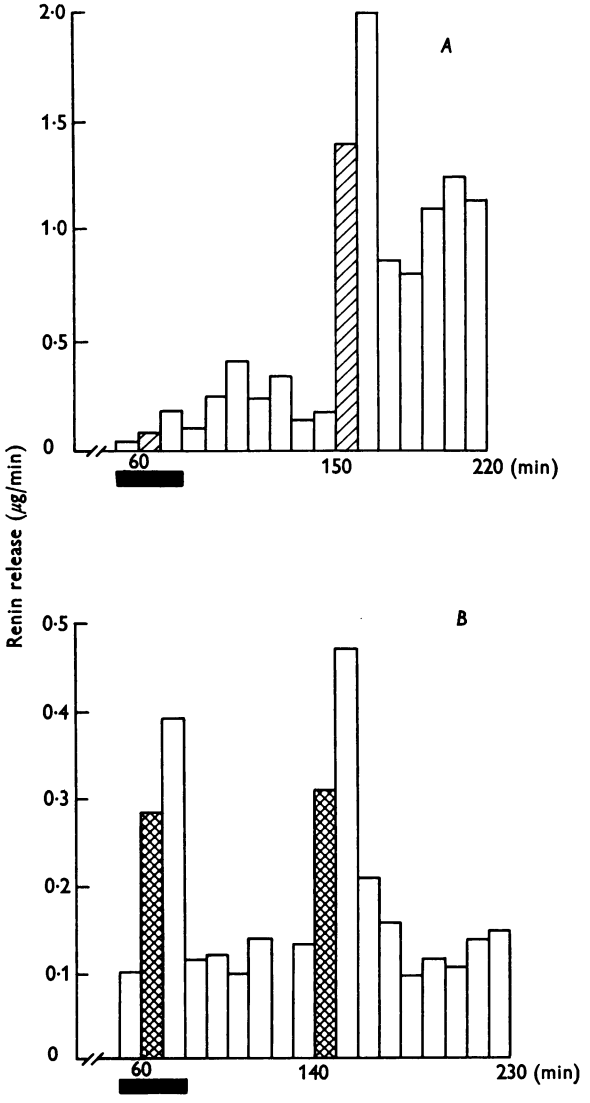

Fig. 2. The effect of propranolol (■) on renin secretion induced by (*A*) isoprenaline or (*B*) glucagon. Kidneys were perfused with Locke solution and propranolol (10 μM) was added during the 50–80th min of perfusion. *A*, isoprenaline (3 μM) was added during the 60–70th and 150–160th min of perfusion. *B*, glucagon (0·3 μM) was added during the 60–70th and 140–150th min of perfusion.

be indicative of a combination of preformed and newly synthesized enzyme, we next investigated the effects of cycloheximide, an inhibitor of protein synthesis, on the secretory response to isoprenaline. Fig. 3*A* shows that the infusion of cycloheximide prior to and during exposure

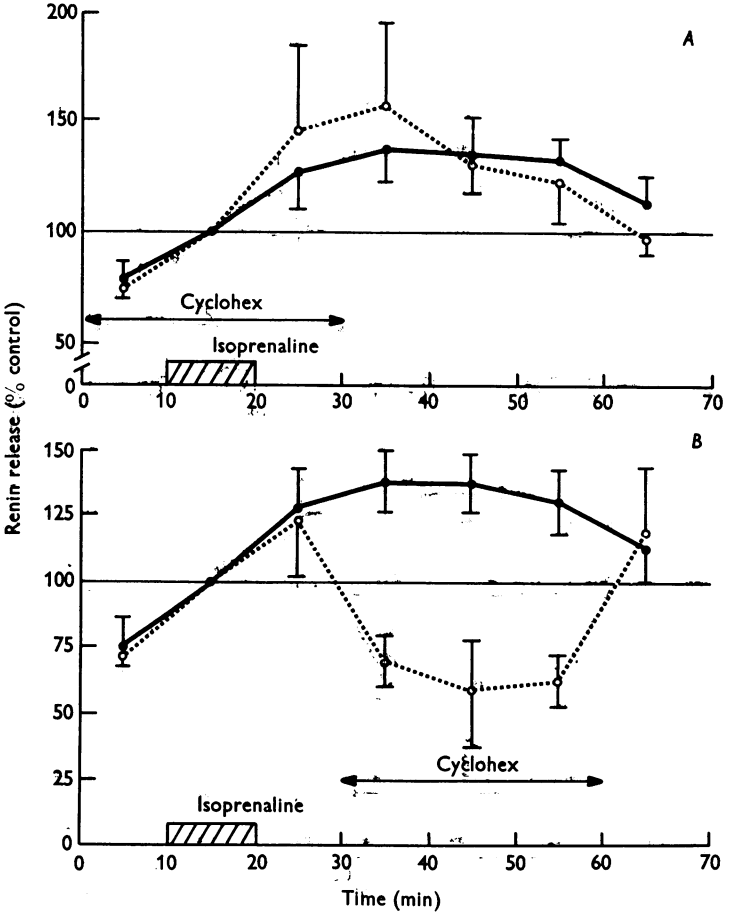

Fig. 3. Cycloheximide action on the time course of isoprenaline-induced renin release. *A*, kidneys were perfused either with normal Locke solution for 70 min or, with Locke solution plus cycloheximide (10 μM) for 30 min followed by normal Locke solution for 40 min. During the 10–20th min of perfusion isoprenaline (0·3 μM) was added. *B*, kidneys were perfused with Locke solution for 70 min and in certain experiments cycloheximide (10 μM) was added during the 30–60th min of perfusion. Isoprenaline was added during the 10–20th min of perfusion. The open and closed circles represent the average rate of renin release (± S.E. of mean) during a given 10 min period from cycloheximide-treated and control kidneys, respectively. Each value was obtained from at least four experiments and is expressed as a percent of the output rate during the 10 min exposure to isoprenaline.

to isoprenaline failed to alter significantly the pattern of the secretory response. By contrast, when the same concentration of cycloheximide was introduced 10 min after isoprenaline, a pronounced fall in the secretion rate was observed, which was rapidly reversed after the inhibitor was removed (Fig. 3*B*).

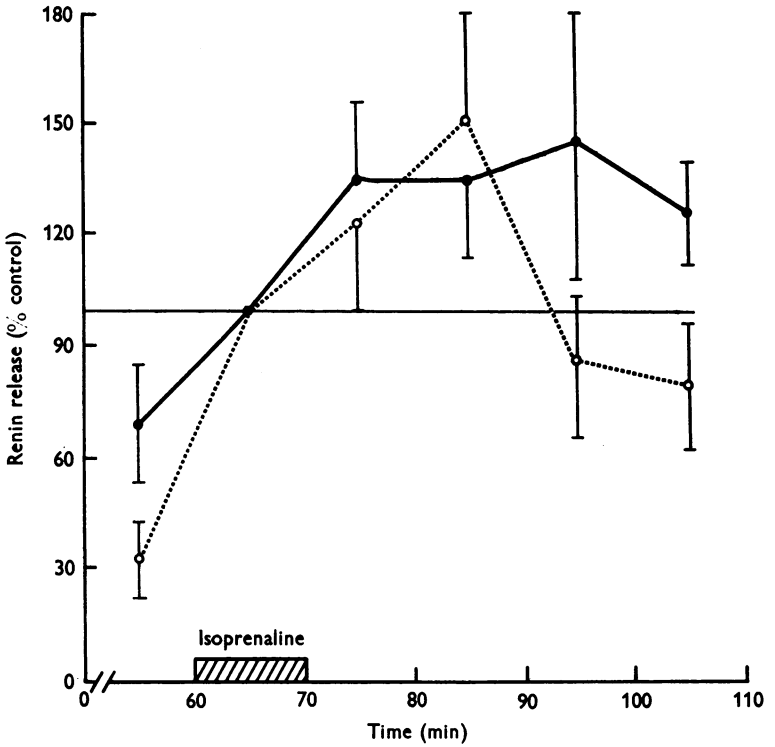

Fig. 4. Comparison of secretory responses to isoprenaline during perfusion with normal and calcium-free media. Kidneys were perfused for 110 min with normal Locke solution or calcium-free Locke plus EGTA. During the 60–70th min of perfusion, isoprenaline was added to the perfusion medium. The closed and open circles represent mean rates of renin secretion during a 10 min collection period in the presence and absence of calcium, respectively. Mean values (± S.E. of mean) were derived from at least four experiments; each is expressed as percent of the output rate during the 10 min exposure to isoprenaline.

The role of calcium in renin secretion

Calcium deprivation. Omission of calcium from the perfusion medium for 60 min failed to depress the early response to isoprenaline. Thus, during the 10 min exposure to isoprenaline, mean renin secretion was augmented 3·3- (± 0·7) and 2·7- (± 0·4) fold in the absence and presence

of calcium, respectively. The early response to isoprenaline was not significantly impaired even after prolonged calcium deprivation; thus, after 120 and 180 min of calcium-free perfusion, isoprenaline-induced secretion was augmented 2·6- (±0·6) and 2·2- (±0·7) fold, respectively.

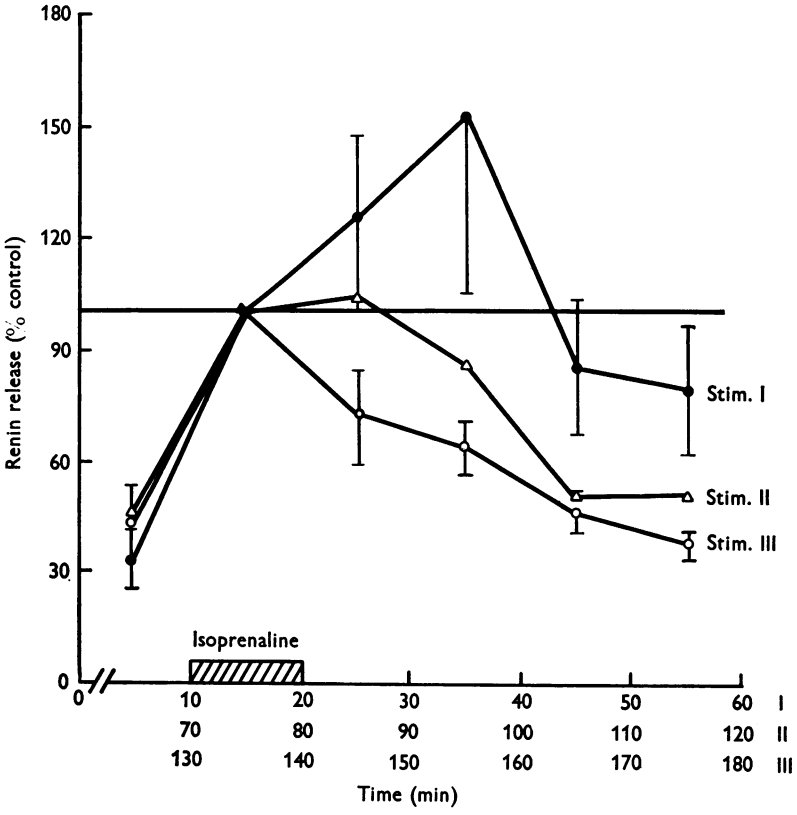

Fig. 5. The effect of calcium lack on the pattern of renin secretion during repeated exposures to isoprenaline. Kidneys perfused with calcium-free Locke solution for 180 min were exposed to isoprenaline during the 10–20th, 70–80th, and 130–140th min of perfusion. The mean values (±S.E. of the mean) (represented as percent of control output rate during the preceding 10 min exposure to isoprenaline) were derived from at least four experiments.

However, closer analysis of the temporal pattern of renin secretion revealed that in the absence of calcium, isoprenaline-induced secretion declined at a more rapid rate (Fig. 4). This rapid decay in the secretory response was more clearly defined during prolonged calcium deprivation with repeated exposures to isoprenaline (Fig. 5). This depressant effect of prolonged calcium deprivation on the late response to isoprenaline was

not the result of a non-specific decrease in the responsivity of the preparation since three successive exposures to the same isoprenaline concentration for only 3 min periods each, which elicited a response of shorter duration, did not produce the enhanced decline in renin secretion during 180 min of calcium-free perfusion. The loss of the later phase of secretion caused by calcium deprivation was, however, irreversible in the sense that restoring calcium to the perfusion medium failed to prolong the response to isoprenaline.

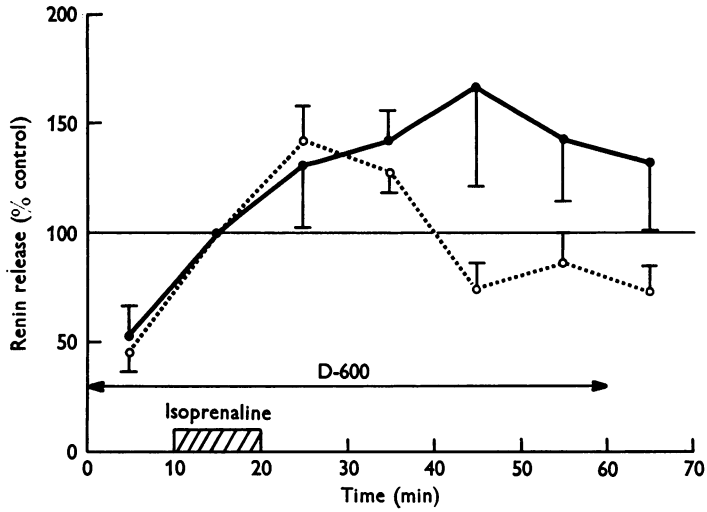

Fig. 6. Influence of D-600 on renin release. Kidneys were perfused with Locke solution in the presence or absence of D-600 (30 μM). During the 10–20th min of perfusion isoprenaline was added. The mean rates of renin secretion ($\pm$ S.E. of mean) in the presence and absence of D-600 are represented by the open and filled circles, respectively and were determined from four experiments. Each value is expressed as a percent of the output rate during the 10 min exposure to isoprenaline.

The effects of D-600. The effects of the calcium antagonist D-600 (α-isopropyl-α(*N*-methyl-*N*-homoveratryl)-γ-aminopropyl-3,4,5-trimethoxyphenyl-acetonitril) on isoprenaline-induced renin secretion were also tested. These experiments were carried out in the presence of 0·5 mM calcium, rather than 2 mM, in order to aid in uncovering any potential inhibitory action of the drug. D-600 failed to depress the early response to isoprenaline; during the 10 min this stimulant was present in the medium, renin release rose 2·4- ($\pm$ 0·5) and 2·6- ($\pm$ 0·5) fold in the absence and presence of D-600, respectively. However, in the presence of D-600, the secretory rate declined more rapidly so that 50 min after isoprenaline stimulation, the secretory rate returned to near basal levels (Fig. 6).

The effect of calcium reintroduction. Following prolonged periods of calcium deprivation, when calcium (2 mM) was restored to the medium, there was a prompt and marked (threefold) enhancement in renin secretion, which gradually declined over the next 30 min (Fig. 7). This stimulant effect of calcium appeared to be a consequence of the prolonged and severe calcium deprivation and not merely the result of an increase in extracellular calcium, since increasing the calcium concentration of the perfusion solution from 0·5 to 2·0 mM failed to elicit any discernible enhancement of renin release.

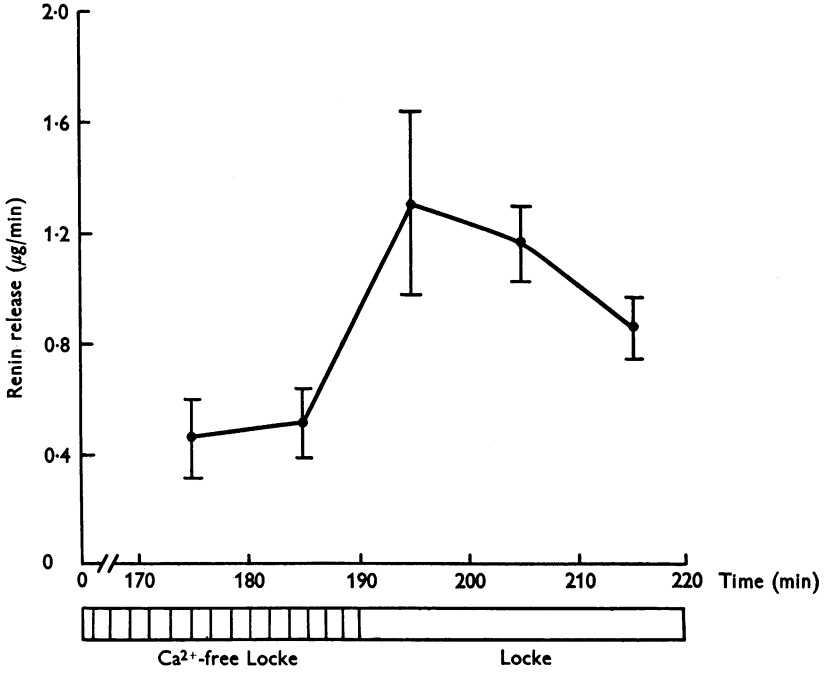

Fig. 7. Renin release by calcium after perfusion with a calcium-free medium. Kidneys were perfused with calcium-free Locke solution plus EGTA for 190 min and with normal Locke solution for an additional 30 min. Each point represents the average rate (± S.E. of mean) of renin output during a 10 min collection period obtained from three experiments.

The stimulant effects of glucagon and furosemide

Although our primary interest concerned renin release induced by isoprenaline, some information was also obtained on secretion elicited by glucagon and furosemide. Glucagon elicited a biphasic pattern of secretion which resembled that of isoprenaline (Fig. 8*A*). The addition of propranolol (10 μM) (Fig. 2*B*) or perfusion with a calcium-free medium failed to diminish the secretory response to glucagon (Fig. 8*A*). Furosemide

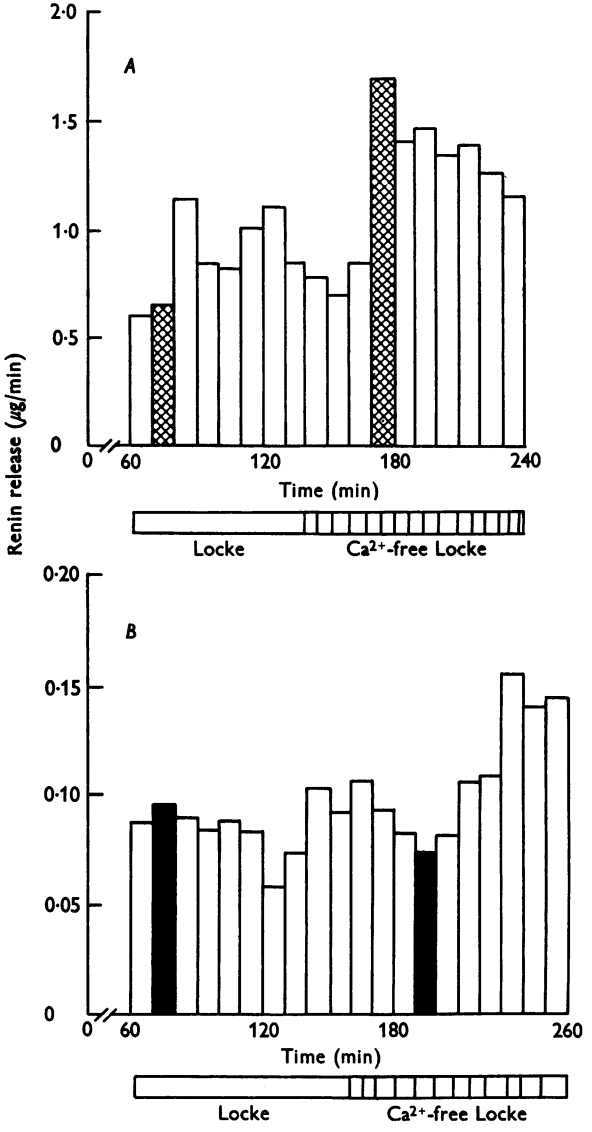

Fig. 8. The effect of calcium deprivation on renin release induced by (*A*) glucagon and (*B*) furosemide. *A*, a kidney was perfused with Locke solution for 140 min and with calcium-free Locke solution for an additional 100 min. During the 70–80th and 170–180th min of perfusion, glucagon (0·3 μM) was added. *B*, a kidney was perfused with Locke solution for 160 min and with calcium-free Locke solution for an additional 100 min. During the 70–80th and 190–200th min furosemide (3 μM) was added. Each vertical bar represents the renin output during a 10 min collection period.

elicited a somewhat different secretion pattern. During a 10 min exposure to this agent, no increase in the secretory rate was observed; the control rate immediately before furosemide addition was 100 ($\pm$ 15)% of the rate during perfusion with the drug (n = 4). The rate slowly increased, reaching a maximum (230 ($\pm$ 66)%) 70 min after furosemide was removed, and then slowly declined. Propranolol (10 μM) was ineffective in depressing furosemide-induced renin release. Calcium-deprivation also failed to impair the secretory response to furosemide and, in fact, may have even enhanced it. Fig. 8*B* depicts an experiment in which the furosemide concentration employed had little or no stimulant effect when given in Locke solution but had a distinct stimulant effect when given in calcium-free Locke solution.

DISCUSSION

Since sympathetic modulation of renin release is exerted through β-adrenergic receptor activation (Ganong, 1973; Vandongen, Peart & Boyd, 1973; Johns, Richards & Singer, 1975), the β-agonist isoprenaline was employed as the major stimulus to study the mechanism of renin secretion by the isolated cat kidney perfused *in situ*. This preparation is exquisitely sensitive to isoprenaline, responding to as little as 0·3 nM of stimulant and eliciting an extended response to higher isoprenaline concentrations. The prolonged response persisting after removal of the stimulus indicates that β-receptor activation induces a relatively persistent change in some component of the juxtaglomerular cell which is responsible for maintaining augmented secretion.

During enhanced release, a biphasic pattern of secretion can be delineated and dissociated with cycloheximide, an inhibitor of protein synthesis. Taken together, these data suggest that isoprenaline enhances release of both pre-formed and newly formed enzyme and that the contribution of the newly formed renin becomes increasingly more important over time. These findings are strikingly similar to those obtained in the endocrine pancreas, where a biphasic pattern of glucose-induced insulin release can be demonstrated (Curry, Bennett & Grodsky, 1968) and only the second phase is blocked by cycloheximide and other inhibitors of protein synthesis (Curry *et al.* 1968; Curry, 1971). These investigators explained the inhibition as not due to an effect on hormone synthesis but rather due to an effect on cellular compartmentalization of the secretory organelles (Grodsky, 1972; Sando & Grodsky, 1973). Although our own evidence does not permit us to distinguish clearly between these alternatives, the rapid reversibility of the depressant action of cycloheximide on renin release suggests that this inhibitor is not interfering with *de novo* synthesis of enzyme but rather with its conversion to an active form or

with its transport to a readily releasable store. Indeed, histochemical and biochemical studies indicate that renin is contained in specific intracellular organelles together with an inactive form called prorenin, which may be a precursor of the releasable enzyme (Edelman & Hartroft, 1961; Morris & Johnston, 1976). Additional morphological evidence for the existence of an early form of a renin-containing secretory granule (protogranule) (Barajas, 1966) further supports the concept that renin is not stored and released as a homogeneous pool and that stimulation of its release involves the interaction of functionally distinct pools or forms of renin.

Interpreting the data from the perfused cat kidney to mean that renin release occurs from heterogeneous compartments is considered an important aspect of this study, since it is necessary to distinguish any effects of calcium directly on the release mechanism from those exerted on synthesis and/or mobilization of secretory product. The finding that prolonged perfusion with a calcium-free medium failed to impair the early response to isoprenaline suggests that extracellular calcium is not required in order to elicit the release response. Although it might be argued that despite perfusion with a calcium-free medium plus chelating agent (EGTA) for 180 min, the relatively high calcium concentrations in the kidney (cf. Rubin, 1974) may contribute sufficient cation to the extracellular milieu to sustain the secretory response, the experiments with D-600 support the idea that transmembrane calcium flux is not a crucial step in the initial response to isoprenaline. D-600, which appears to block specific calcium channels in the cell membrane (Kohlhardt, Bauer, Krause & Fleckenstein, 1972), antagonizes hormone release in such systems as the adrenal medulla (Pinto & Trifaro, 1976) and neurohypophysis (Russell & Thorn, 1974) where extracellular calcium plays a major role in the secretory process. In the present investigation D-600, while blocking the late phase, failed to depress the early phase of isoprenaline-induced renin secretion, lending further support for the idea that if an early signal generated by β-receptor activation is an influx of extracellular calcium, it is not primarily responsible for promoting renin secretion. Precedent for this type of mechanism can be found in the rat parotid gland, where isoprenaline-induced amylase release by β-receptor activation does not appear to directly involve extracellular calcium (cf. Selinger, 1975). In this gland, calcium is thought to participate at some later stage of the sequence of events associated with the action of cyclic AMP, rather than as the primary messenger.

Since it is generally accepted that the effects of β-receptor activation are mediated through the adenylate cyclase–cyclic AMP system (Robison, Butcher & Sutherland, 1971), it is not surprising that evidence exists for the participation of cyclic AMP in renin release (Michelakis, Caudle &

Liddle, 1969; Winer, Chokshi & Walkenhorst, 1971). Certain effects of glucagon, including the induction of renin secretion (Vandongen *et al.* 1973), although not associated with β-receptor activation, are similar to the effects of isoprenaline and are associated with alterations in cyclic AMP levels (cf. Robison *et al.* 1971). The fact that the stimulant action of glucagon on renin release is also not dependent upon extracellular calcium implies that this refractoriness to extracellular calcium is not inextricably linked to β-receptor activation. The renin releasing activity of furosemide is not a consequence of its diuretic action since it can induce secretory activity in isolated renal cortical cells (Lyons & Churchill, 1975) and it does not appear to involve activation of β-receptors or mediation by cyclic AMP (Johns & Singer, 1973). The diverse pattern of furosemide-induced renin release observed in the perfused cat kidney also suggests a mechanism of action which is different from that of isoprenaline and glucagon. Since furosemide was also an effective secretagogue during calcium-free perfusion, the dispensability of extracellular calcium in renin secretion thus appears to transcend the mode of stimulation.

The second phase of renin secretion could be dissociated not only by its sensitivity to cycloheximide but also by its dependence on extracellular calcium. Both calcium-free perfusion and the addition of D-600 depressed the second phase of secretion elicited by isoprenaline, suggesting that calcium influx into the juxtaglomerular cell occurs during membrane activation. Since this phase is cycloheximide-sensitive, the entry of calcium must be concerned with certain of the events which follow secretion, such as synthesis and/or mobilization of secretory product.

Although these experiments have seemingly demonstrated that extracellular calcium may not be required for renin secretion, they by no means imply that the intracellular receptor responsible for triggering the release of this enzyme is not calcium-sensitive. Indeed, the finding that calcium was capable of directly stimulating renin release following a period of calcium free perfusion provides strong support for the view that calcium acts as the mediator linking stimulation to renin secretion, just as it does in a variety of other secretory systems, such as the adrenal medulla, where release occurs by exocytosis (Smith & Winkler, 1972). This finding is interpreted in a manner identical with that proposed for a similar result obtained in the adrenal medulla, where the reintroduction of calcium following a period of calcium deprivation causes an explosive but transient increase in catecholamine release (Douglas & Rubin, 1961, 1963). It was suggested that in the absence of calcium, membrane permeability increases and that added calcium readily penetrates these leaky cells and triggers secretion (cf. Douglas, 1975).

The fundamental concept of the role of calcium in stimulus-secretion

coupling affirms that an increase in intracellular calcium ion is primarily responsible for activating the secretory process (Rubin, 1970, 1974; Douglas, 1975); but it should again be emphasized that the increase in free cellular cation can be brought about by mobilization of intracellular calcium stores as well as by an influx of extracellular calcium (Rubin, 1974).

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REFERENCES

BARAJAS, L. (1966). The development and ultrastructure of the juxtaglomerular cell granule. *J. Ultrastruct. Res.* **15**, 400–413.

CALDWELL, B. V., BURSTEIN, S., BROCK, W. A. & SPEROFF, L. (1971). Radioimmunoassay of the F prostaglandins. *J. clin. Endocr. Metab.* **33**, 171–175.

CHEN, D. S. & POISNER, A. M. (1976). Direct stimulation of renin release by calcium. *Proc. Soc. exp. Biol. Med.* **152**, 565–567.

CURRY, D. L. (1971). The effect of cycloheximide and 2-deoxy-glucose on the diphasic pattern of insulin secretion. *Acta diabetolog. latina* **8**, 48–65.

CURRY, D. L., BENNETT, L. L. & GRODSKY, G. M. (1968). Dynamics of insulin secretion by the perfused rat pancreas. *Endocrinology* **83**, 572–584.

DAVIS, J. O. & FREEMAN, R. H. (1976). Mechanisms regulating renin release. *Physiol. Rev.* **56**, 1–56.

DOUGLAS, W. W. (1975). Secretomotor control of adrenal medullary secretion: synaptic, membrane, and ionic events in stimulus-secretion coupling. In *Handbook of Physiology*, vol. 6, section 7, ed. BLASCHKO, H., SAYERS, G. & SMITH, A. D., pp. 367–388. Baltimore: Williams and Wilkins.

DOUGLAS, W. W. & RUBIN, R. P. (1961). The role of calcium in the secretory response of the adrenal medulla to acetylcholine. *J. Physiol.* **159**, 40–57.

DOUGLAS, W. W. & RUBIN, R. P. (1963). The mechanism of catecholamine release from the adrenal medulla and the role of calcium in stimulus-secretion coupling. *J. Physiol.* **167**, 288–310.

EDELMAN, R. & HARTROFT, P. M. (1961). Localization of renin in juxtaglomerular cells of rabbit and dog through the use of the fluorescent antibody technique. *Circulation Res.* **9**, 1069–1077.

GANONG, W. F. (1973). Biogenic amines, sympathetic nerves, and renin secretion. *Fedn Proc.* **32**, 1782–1784.

GRODSKY, G. M. (1972). A threshold distribution hypothesis for packet storage of insulin. II. Effect of calcium. *Diabetes* **21**, suppl. 2, 584–593.

HABER, E., KOERNER, T., PAGE, L. B., KLIMAN, B. & PURNODE, A. (1969). Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. *J. clin. Endocr. Metab.* **29**, 1349–1355.

IWAO, H., ABE, Y. & YAMAMOTO, K. (1974). Effect of intrarenal arterial infusion of calcium on renin release in dogs. *Jap. J. Pharmac.* **24**, 482–484.

JOHNS, E. J., RICHARDS, H. K. & SINGER, B. (1975). Effects of adrenaline, noradrenaline, isoprenaline and salbutamol on the production and release of renin by isolated renal cortical cells of the cat. *Br. J. Pharmac.* **53**, 67–73.

JOHNS, E. J. & SINGER, B. (1973). Effect of propranolol and theophylline on renin release caused by furosemide in the cat. *Eur. J. Pharmac.* **23**, 67–73.

KOHLHARDT, M., BAUER, B., KRAUSE, H. & FLECKENSTEIN, A. (1972). Differentiation of the transmembrane Na and Ca channels in mammalian cardiac fibers by the use of specific inhibitors. *Pflügers Arch.* **335**, 309–322.

KOTCHEN, T. A., MAUL, K. I., LUKE, R., REES, D. & FLAMENBAUM, W. (1974). Effect of acute and chronic calcium administration on plasma renin. *J. clin. Invest.* **54**, 1279–1286.

LESTER, G. E. & RUBIN, R. P. (1976). Dynamics of renin release from the perfused cat kidney. *Pharmacologist* **18**, 151.

LYONS, H. J. & CHURCHILL, P. C. (1975). Renin secretion from rat renal cortical cell suspensions. *Am. J. Physiol.* **228**, 1835–1839.

MICHELAKIS, A. M. (1971). The effect of Na and Ca on renin release *in vitro*. *Proc. Soc. exp. Biol. Med.* **137**, 833–836.

MICHELAKIS, A. M., CAUDLE, J. & LIDDLE, G. W. (1969). *In vitro* stimulation of renin production by epinephrine, norepinephrine and cyclic AMP. *Proc. Soc. exp. Biol. Med.* **130**, 748–753.

MORIMOTO, S. K., YAMAMOTO, K., HORIUCHI, K., TANAKA, H. & UEDA, J. (1970). A release of renin from dog kidney cortex slices. *Jap. J. Pharmac.* **20**, 536–545.

MORRIS, B. J. & JOHNSTON, C. I. (1976). Isolation of renin granules from rat kidney cortex and evidence for an inactive form of renin (prorenin) in granules and plasma. *Endocrinology* **98**, 1466–1474.

PINTO, J. E. B. & TRIFARO, J. M. (1976). The effects of D-600 (methoxyverapamil) on the release of adrenal catecholamines induced by acetylcholine, high potassium or sodium deprivation. *Br. J. Pharmac.* **57**, 127–132.

ROBISON, G. A., BUTCHER, R. W. & SUTHERLAND, E. W. (1971). *Cyclic AMP*, pp. 145–270. London: Academic Press.

RUBIN, R. P. (1970). The role of calcium in the release of neurotransmitter substances and hormones. *Pharmac. Rev.* **22**, 389–428.

RUBIN, R. P. (1974). *Calcium and the Secretory Process*, pp. 25–124. New York: Plenum.

RUSSELL, J. T. & THORN, N. A. (1974). Calcium and stimulus-secretion coupling in the neurohypophysis. II. Effects of lanthanum, a verapamil analogue (D-600) and prenylamine on 45-calcium transport and vasopressin release in isolated rat neurohypophyses. *Acta endocr., Copenh.* **76**, 471–487.

SANDO, H. & GRODSKY, G. M. (1973). Dynamic synthesis and release of insulin and proinsulin from perifused islets. *Diabetes* **22**, 354–360.

SELINGER, Z. (1975). Diverse functions of calcium in the rat parotid acinar cell. In *Calcium Transport in Contraction and Secretion*, ed. CARAFOLI, E., CLEMENTI, F., DRABIKOWSKI, W. & MARGRETH, A., pp. 139–146. Amsterdam: North-Holland.

SMITH, A. D. & WINKLER, H. (1972). Fundamental mechanisms in the release of catecholamines. In *Handbook of Pharmacology*, chap. 33, ed. BLASCHKO, H. & MUSCHOLL, E., pp. 546–565. Berlin: Springer.

THURAU, K., SCHNERMANN, J., NAGEL, W., HORSTER, M. & WOHL, M. (1967). Composition of tubular fluid in the macula densa segment as a factor regulating the function of the juxtaglomerular apparatus. *Circulation Res.* **21**, suppl. 2, 79–89.

VANDER, A. J. (1967). Control of renin release. *Physiol. Rev.* **47**, 359–382.

VANDER, A. J. (1970). Direct effects of potassium on renin secretion and renal function. *Am. J. Physiol.* **219**, 455–459.

VANDONGEN, R., PEART, W. S. & BOYD, G. W. (1973). Adrenergic stimulation of renin secretion in the isolated perfused kidney. *Circulation Res.* **32**, 290–296.

WINER, N., CHOKSHI, D. S. & WALKENHORST, W. G. (1971). Effect of cyclic AMP, sympathomimetic amines and adrenergic receptor antagonist on renin secretion. *Circulation Res.* **29**, 239–248.