J. Physiol. (1981), **310**, *pp.* 285–292
With 5 text-figures
Printed in Great Britain

RENIN RELEASE FROM ISOLATED RAT GLOMERULI: SEASONAL VARIATIONS AND EFFECTS OF D600 ON THE RESPONSE TO CALCIUM DEPRIVATION

BY LARS BAUMBACH AND OLE SKØTT

From the University Institute for Experimental Medicine, 71 Nørre Allé, 2100 Copenhagen Ø, Denmark

(*Received 26 March 1980*)

SUMMARY

1. The effects of calcium deprivation and D600 on the rate of renin release and seasonal variations in the response were studied on juxtaglomerular cells from a preparation of isolated rat glomeruli superfused *in vitro*.

2. Reduction of superfusate calcium concentration caused an increase in renin release, which was significantly higher during the summer (May–August) than during the rest of the year.

3. Addition of D600 (2×10^{-4} M) to a calcium-free medium in the low responsive period caused a markedly increased renin release. In the high responsive period renin release increased more rapidly and to a higher level initially than observed in the control lines without D660.

4. It is suggested that the effect of calcium on renin release predominantly is mediated by changes in calcium bound to the plasma membrane of the juxtaglomerular cell. The sensitivity of this cell to changes in the extracellular calcium concentration seems to be regulated and varies with season, possibly due to regulation of the amount of calcium bound to the membrane.

INTRODUCTION

The effects of calcium on renin release have been investigated in extensive series of experiments performed on various preparations and the results and conclusions have apparently been contradictory. Thus, acute exposure to a calcium-free extracellular fluid causes an increase in renin release both from the isolated perfused intact kidney and from cortical slices and isolated glomeruli *in vitro* (Vandongen & Peart 1974; Baumbach & Leyssac 1977; Fray 1977; Harada, Lester & Rubin 1979). However a transient increase in renin release following addition of calcium to the perfusate after prolonged exposure to calcium-free medium has also been reported (Lester & Rubin 1977; Chen & Poisner 1976). Obviously the effects and mechanisms of action of calcium on renin release are poorly understood.

In an attempt to define more precisely the role of calcium in renin release, we have used a preparation of isolated rat glomeruli (Blendstrup, Leyssac, Poulsen & Skinner, 1975) and a calcium transport inhibitor, D600 (methoxyverapamil) belonging to a

0022-3751/81/3300-0887 $07.50

group of agents initially described by Hass & Härtfelder (1962). Initial results showed that the response of the preparation varied according to the time of year; we also therefore present data on seasonal variation over the years 1975–1980.

METHODS

Male Sprague–Dawley rats (250–350 g) with free access to ordinary rat chow (Rostock, KFK, Denmark) and tap water were anaesthesized with Amytal® (sodium amobarbitone). Batches of 300 microscopically selected rat glomeruli (five batches per rat) were prepared by the magnetic iron oxide technique of Cook & Pickering (1959) and superfused with Ringer solutions in five polyethylene lines as previously described (Blendstrup *et al.* 1975). The glomeruli with attached juxtaglomerular cells were held in the superfusion lines by a magnetic field (0, 0030 Wb) during superfusion at a rate of 10 μl./min from one of two infusion pumps (Braun). This arrangement permitted abrupt changes of superfusate composition during an experiment without disturbing the glomeruli, and allowed each line to serve as its own control. In addition two or three of the five lines served as controls throughout the experiment.

The glomeruli were prepared in and superfused during control periods with a bicarbonate Ringer solution, the composition of which was (m-mole/l.): NaCl, 101·00; $NaHCO_3$, 17·5; KCl, 7·0; $CaCl_2$, 2·0; $MgSO_4$, 1·2; NaH_2PO_4. 1·2; glucose, 11·0; and sucrose, 34·0; giving a calculated osmolality of 305 m-osmole/kg. The Ringer was adjusted to pH 7·4 by bubbling with 5% CO_2 and 95% O_2 at 37 °C. Molal solute concentrations were checked on a semimicro osmometer (Knauer). In the calcium-free buffers no corrections were made in the composition of the Ringer solution to allow for the small change in osmolality.

D600 (α-isopropyl-α-((*N*-methyl-*N*-homoveratryl)-γ-aminopropyl)-3,4,5-trimethoxy-phenyl-acetonitril i.e. methoxyverapamil) was added directly to buffers to give final concentrations of 2×10^{-4} and 2×10^{-5} M respectively. When ethylene-glycol-2-(2-aminoethyl)-tetracetic acid (EGTA) was added to the calcium-free superfusion buffers, a final concentration of 0·5 mM-EGTA was used. Superfusate was collected over 12 min periods and renin assayed in 25 μl. volumes by radioimmunoassay of generated angiotensin I using the 'trapping' technique of Poulsen & Jørgensen (1974). Detection limit of the assay is 10^{-7} Goldblatt units (GU) contained in 25 μl. The remaining renin content of the batches at the end of the experiment was assayed after extraction by freezing and thawing three times (Blendstrup *et al.* 1975). Renin is expressed in terms of Goldblatt hog units (M.R.C. Holly Hill, London).

All experiments were performed at a temperature of 30 °C. When the glomeruli were exposed to changes in superfusate composition these changes were introduced after approximately 50 min of superfusion with the control buffer, and the moment of change is referred to as zero time.

Statistical analysis

The absolute value of renin release in the last control period preceding the zero time was used as the control value for the individual experiment and ascribed the value of 100%. Significance of changes in renin release were estimated by Student's *t* test.

RESULTS

Seasonal changes in renin release response to calcium deprivation

Fig. 1 is a mass graph ($n = 93$) of the level of renin release after 36 min of calcium deprivation obtained in each calendar month over the period 1975–1980. It is apparent that the renin release response to calcium deprivation varies with the season being high from late April to mid August (high responsive period) and low during the rest of the year (low responsive period).

Fig. 2 gives mean values of renin release responses to long term calcium deprivation obtained in 1979. The data have been separated into two groups according to the two periods appearing from Fig. 1. After 72 min of calcium deprivation, renin release had increased to 700% in the high responsive period, and only to 125% in the low

responsive period. The difference is highly significant ($P < 0{\cdot}001$). Both renin release levels are significantly higher ($P < 0{\cdot}001$) than control experiments containing 2 mM-calcium (57 % of zero value after 72 min).

Addition of EGTA (0·5 mM) in the low responsive period had no effects on the renin release response to calcium deprivation.

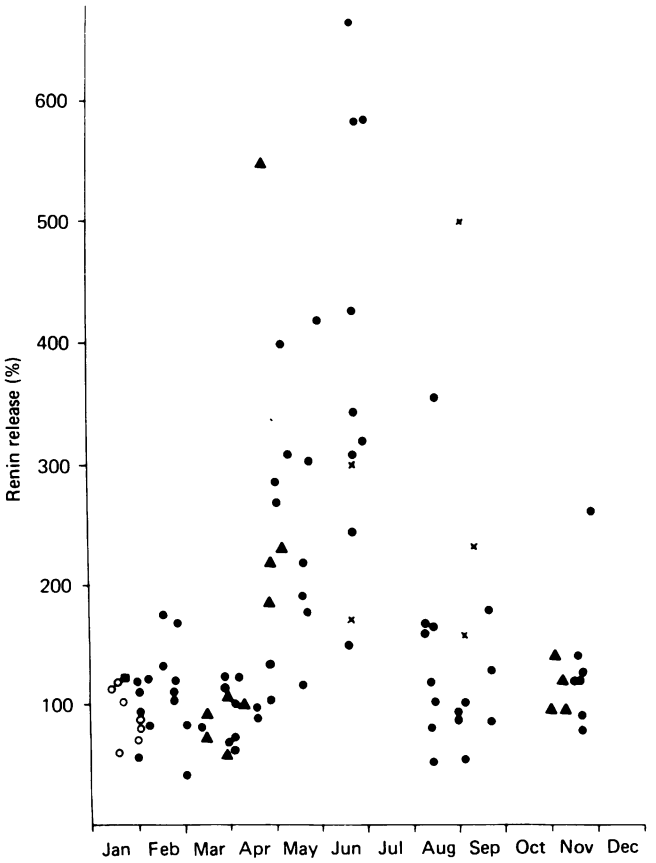

Fig. 1. Mass graph of renin release measured after 36 min of calcium deprivation plotted against the calendar months (× = 1975, ▲ = 1976, ○ = 1977, ● = 1979, ■ = 1980, $n = 93$).

Resting renin release (the absolute value of the 100 % level) during the high responsive period was not significantly different from that in the low responsive period ($1{\cdot}2 \pm 0{\cdot}2$ ($n = 25$) and $1{\cdot}5 \pm 0{\cdot}3$ ($n = 34$) $\times 10^{-7}$ GU/100 glomeruli. min. respectively). Neither did the total renin content of the glomeruli differ (3·2 ($n = 25$) and 3·3 ($n = 34$) $\times 10^{-4}$ GU/100 glomeruli respectively) in the high and low responsive periods. The percentage of the total renin content released during 72 min of calcium deprivation was 13 % in the summer and 4 % in the remaining part of the year.

The renin release pattern in control experiments (2 mM-calcium) was identical throughout the year and not statistically different from previous experiments; all control experiments are therefore pooled for presentation (filled squares, Fig. 2).

Neither the technique of preparation, nor the composition of the food, nor the content of salts in the drinking water has been changed during the year.

Effects of D600

a. Effects of 2×10^{-5} *M D600.* 2×10^{-5} D600 had no effect on renin release either when added to a 2 mM-calcium superfusate ($n = 6$, $P > 0{\cdot}05$), or to a calcium-free superfusate (low responsive period $n = 6$, $P > 0{\cdot}05$; high responsive period $n = 9$, $P > 0{\cdot}05$), or when EGTA (0·5 mM) was added to the calcium-free medium ($n = 8$, $P > 0{\cdot}05$) in the low responsive period.

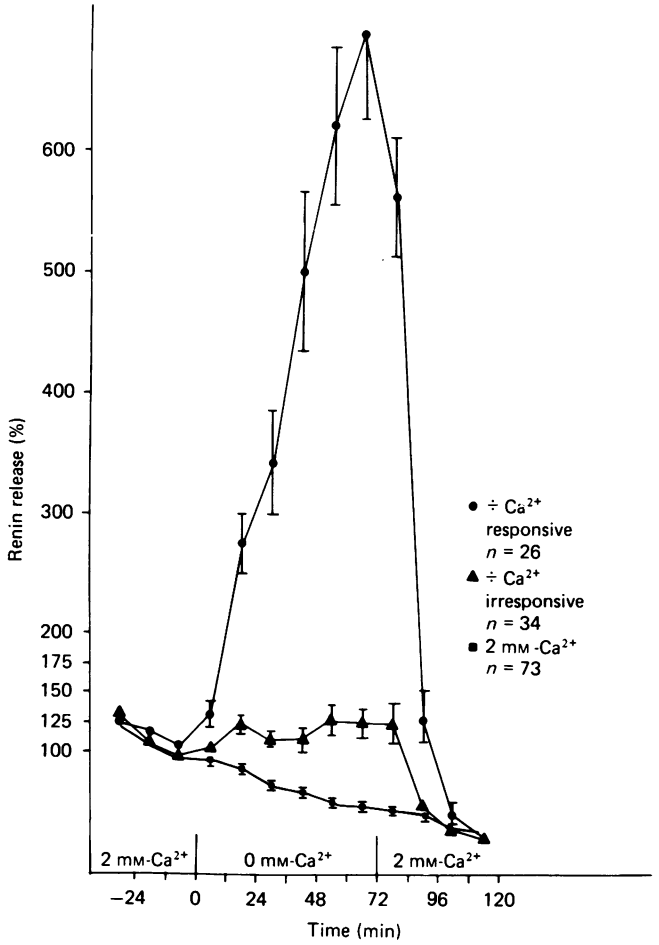

Fig. 2. Effect of calcium deprivation during the high responsive period (●, $n = 26$) and the low responsive period (▲, $n = 34$). All data obtained in 1979. Lowest line represents control experiments containing 2 mM-calcium (■, $n = 73$). Bars indicate ±S.E. of mean.

b. Effects of 2×10^{-4} M *D600.* When added to a 2 mM-calcium medium D600 did not change renin release significantly ($n = 6$, $P > 0{\cdot}05$).

During the high responsive period addition of D600 to a calcium free superfusate increased the initial acceleration of renin release significantly above that of controls

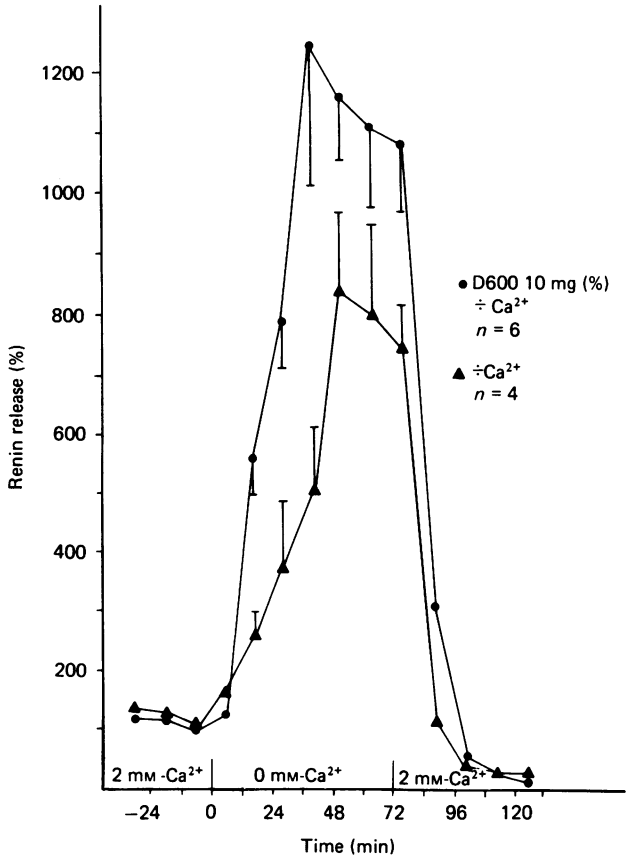

Fig. 3. Effects of D600 (2×10^{-4} M) (●, $n = 6$) on renin release stimulated by calcium-free medium (▲, $n = 4$) during high responsive period. Bars indicate S.E. of mean.

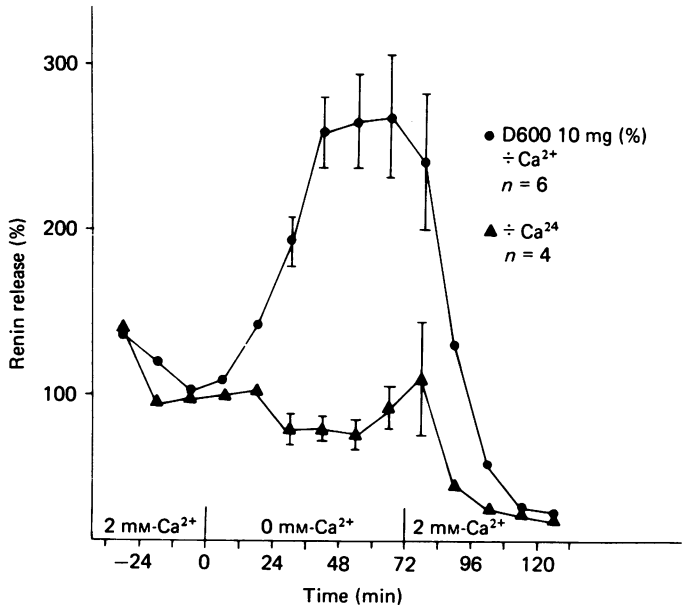

Fig. 4. Effects, of D600 (2×10^{-4} M) (●, $n = 6$) on renin release stimulated by calcium-free medium (▲, $n = 4$) during low responsive period. Bars indicate ±S.E. of mean.

($P < 0{\cdot}02$), whereas there was no significant difference in the level of renin release between glomeruli exposed to D600 and controls after 48 min of superfusion with calcium free medium (Fig. 3).

During the low responsive period addition of D600 to a calcium free superfusate increased renin release significantly above that of the control lines over the entire period of calcium deprivation ($P < 0{\cdot}001$, Fig. 4).

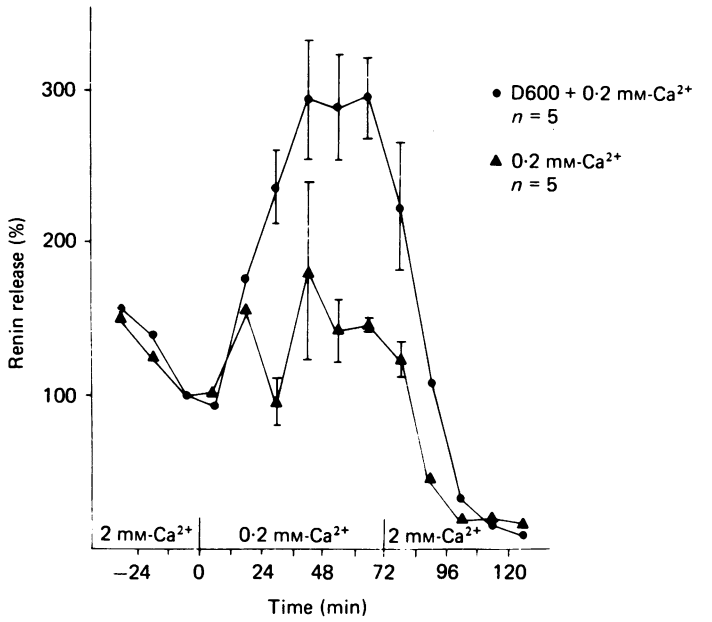

Fig. 5. Effect of D600 (2×10^{-4} M) (●, $n = 5$) on renin release stimulated by lowering calcium concentration to 0·2 mM (▲, $n = 5$). Bars indicate ±S.E. of mean.

In order to exclude the presence of non-specific destruction of the cells by calcium deprivation (Harada *et al.* 1979), a few experiments using 0·2 mM-calcium were performed. In this medium D600 increased renin release significantly above that of the control lines without D600 ($P < 0{\cdot}001$). These results were obtained in August (Fig. 5). All renin release responses were reversible.

DISCUSSION

A reversible increase in the rate of renin release was found in response to a reduction in the calcium concentration of the superfusate. However, when all data on calcium deprivation from the years 1975–1980 were considered, it appeared that seasonal factor(s) had a decisive influence on the magnitude of the renin release response; the response during the summer months was much higher (high responsive period) than during the rest of the year (low responsive period). The seasonal variations could hardly be the result of insufficient calcium deprivation during the low responsive period, since addition of EGTA did not change a low responsive preparation into a

high responsive one. The reasons for the seasonal variation are not known. Although non-detected variations in food intake may be responsible, this is by no means certain.

D600 had no effect in a 2 mM-calcium superfusate in our preparation, probably because calcium inhibits the effect of D600 (Fleckenstein 1971). At low external calcium concentrations of 0·2 mM or less, D600 consistently stimulated renin release significantly.

A decrease in the extracellular calcium concentration has been postulated to induce an increased efflux of calcium from the cell, thereby decreasing the intracellular free calcium ion concentration and consequently increasing renin release (Peart 1978; Fray 1977). Assuming the effect of D600 to be exclusively an inhibition of calcium fluxes, D600 would be expected to inhibit the efflux of calcium after lowering the external calcium concentration. Accordingly D600 ought to diminish the renin release response to calcium deprivation. The present results, in fact, show the opposite.

Alternatively, a reduction of calcium efflux by D600 under calcium free conditions might reduce the supply of calcium to the outside of the plasma membrane from intracellular stores. In this case the observed augmented response to calcium deprivation would be compatible with the hypothesis that a reduction in calcium bound to the membrane is the major stimulating factor, as is also suggested from previous data on the effect of lanthanum (Baumbach & Leyssac 1977). Circumstantial support for this hypothesis is the fact that D600 was only effective at concentrations exceeding 10^{-4} M in our preparation. This suggests that possible effects of D600 other than that on calcium fluxes should be taken into consideration. Membrane fractions from smooth vascular muscle will have their capability of binding ^{45}Ca reduced when exposed to concentrations of verapamil greater than 10^{-5} M (Thorens & Hauesler 1979). Isolated membranes from heart muscle also have a reduced capacity to bind calcium after treatment with verapamil (Nayler, Dunnett & Sullivan 1976). Thus, the increase in renin release response to calcium free medium in the presence of D600 might well be the result of a direct decrease in calcium bound to the membrane.

D600 caused less difference between experimental lines and control lines during the high responsive period than during the low responsive period. According to the above interpretation, this would suggest that the juxtaglomerular cell is less sensitive to removal of membrane-bound calcium during the high responsive period than during the low responsive period. If that is true, the seasonal variations in renin release response would suggest that membrane bound calcium is a parameter regulated with change of season.

Increased renin release due to a decrease in membrane bound calcium does not necessarily contradict previous results (Baumbach & Leyssac, 1977) which showed that addition of the ionophore A23187 to a calcium free medium reduced renin release. This reduction could be due to an increased delivery of calcium from intracellular stores to the outside, counteracting the decrease of membrane-bound calcium.

In summary, results with D600 suggest that calcium bound to the membrane of the juxtaglomerular cell is important in the release of renin, and that it varies with the season. The results do not exclude the possibility that intracellular calcium ion concentration might also affect renin release by other mechanisms.

The present work was supported by grants from the Danish Medical Research Council and the Danish Heart Association. The authors are indebted to Dr Leyssac for valuable advice and criticism throughout this project. The skilful technical assistance of Conni Temdrup and Vibeke Meyland-Smith is acknowledged.

REFERENCES

BAUMBACH L. & LEYSSAC, P. P. (1977). Studies on the mechanism of renin release from isolated superfused rat glomeruli: Effects of calcium, calcium ionophore and lanthanum. *J. Physiol.* **273**, 745–764.

BLENDSTRUP, K. LEYSSAC, P. P., POULSEN K. & SKINNER, S. L. (1975). Characteristics of renin release from isolated superfused glomeruli in vitro. *J. Physiol.* **246**, 653–672.

CHEN, D. S. & POISNER, A. M. (1975). Direct stimulation of renin release by calcium. *Proc. Soc. exp. Biol. Med.* **152**, 565–567.

COOK W. F. & PICKERING, G. W. (1959). The location of renin in rabbit kidney. *J. Physiol.* **149**, 526–536.

FLECKENSTEIN A. (1971). Specific inhibitors and promotors of calcium action in the excitation-contraction coupling of heart muscle and their role in the prevention or production of myocardial lesions. *In Calcium and the Heart*, pp. 135–188, ed. HARRIS, P. & OPIE, J. H. London: Academic Press.

FRAY, J. C. S. (1977). Stimulation of renin release in perfused kidney by low calcium and high magnesium. *Am. J. Physiol* **232**, F377-F382.

HAAS, H. & HÄRTFELDER, G. (1962). α-isopropyl-α-((*N*-methyl-*N*-homoveratryl)-γ-amino-propyl)-3,4-dimethoxyphenyl-acetonitril, eine substanz mit coronar-gefäss-erweiternden eigenschaften. *Arzneim.-Forsch.* **12**, 549–558.

HARADA, E., LESTER, G. E. & RUBIN, R. E. (1979). Stimulation of renin secretion from the intact kidney and from isolated glomeruli by the calcium ionophore A23187. *Biochim. biophys. Acta.* **583**, 20–27.

LESTER, G. E. & RUBIN, R. P. (1977). The role of calcium in renin secretion from the isolated perfused cat kidney. *J. Physiol.* **269**, 93–108.

NAYLER, W. G., DUNNETT, J. & SULLIVAN, A. (1976). Drug-induced changes in the superficially located stores of calcium in heart sarcolemma. In *The Sarcolemma*, ed. ROY P. E. & DHALLA, N. S., pp. 53–70. Baltimore: University Park Press.

PEART, W. S. (1978). Renin 1978. *Johns Hopkins med. J.* **143**, 193–206.

POULSEN, K. & JØRGENSEN J. (1974). An easy radioimmunological microassay of renin activity, concentration and substrate in human and animal plasma and tissue based on angiotensin-I trapping by antibody. *J. Clin. endocr. Metab.* **39**, 764–773.

THORENS, S. & HAUESLER, G. (1979). Effects of some vasodilators on calcium translocation in intact and fractionated vascular smooth muscle. *Eur. J. Pharmacol.* **54**, 79–91.

VANDONGEN R. & PEART, W. S. (1974). Calcium dependence of the inhibitory effect of angiotensin on renin secretion in the isolated perfused kidney of the rat. *Br. J. Pharmac.* **50**, 125–129.