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CALCIUM DEPENDENCE OF THE INHIBITORY EFFECT OF ANGIOTENSIN ON RENIN SECRETION IN THE ISOLATED PERFUSED KIDNEY OF THE RAT

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1 The effect of calcium on the inhibition of renin secretion by biologically active angiotensin was investigated in the isolated rat kidney perfused with Krebs-Ringer saline.

2 In the presence of calcium (3.7 mM), asp(NH_2)^1-angiotensin II suppressed both basal and isoprenaline-stimulated renin secretion. Renal perfusion pressure, which was increased by the infusion of angiotensin, returned to control levels when isoprenaline was added.

3 When the calcium concentration was reduced to 0.32 mM, the vasoconstriction produced by angiotensin was abolished although the inhibitory effect on renin secretion was still evident.

4 In the absence of calcium, angiotensin no longer suppressed basal renin secretion and a prompt increase in renin secretion occurred when isoprenaline was added.

5 The higher basal renin levels which were observed in calcium-free perfusions, suggest the existence of an intrarenal calcium-dependent mechanism that regulates basal renin secretion.

6 These observations indicate that the inhibitory effect of biologically active angiotensin, on basal and isoprenaline-stimulated renin secretion, is functionally related to the contractor response by its dependence on calcium. The recognition that the renin-producing cells are modified smooth muscle cells supports this association

Introduction

It has been shown that renin secretion in the isolated rat kidney, following stimulation with isoprenaline (van Dongen, Peart & Boyd, 1973a), is suppressed by biologically active angiotensin (van Dongen, Peart & Boyd, 1973b). The suppression of renin secretion, which is dissociated from the renal vasoconstrictor effect of angiotensin, is nevertheless dependent on the same structural determinants of the angiotensin molecule necessary for pressor and constrictor activity.

In common with other vasoconstrictors (Somlyo & Somlyo, 1968), the contractor response of smooth muscle to angiotensin is at least partly dependent on the entry of calcium into the cell (Sullivan & Briggs, 1968). Free intracellular calcium may also be increased by the interaction of angiotensin with its intracellular microsomal receptors, which results in the release of bound calcium (Baudouin, Meyer, Fermandjian & Morgat, 1972). Since the inhibition of renin secretion is observed only with biologically active angiotensin, it is conceivable that this effect is mediated by a similar calcium-dependent mechanism. The present study was undertaken to determine the effect of calcium on the renin-inhibitory and renal vasoconstrictor actions of angiotensin in the isolated kidney preparation.

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Methods

Kidney perfusion

Male Wistar rats (300-400 g), maintained on a regular diet were anaesthetized with sodium pentobarbitone (0.1 mg/g i.m.) and injected with heparin (50-100 units i.v.). The left kidney was isolated and perfused without interruption of blood flow as previously described (van Dongen *et al.*, 1973a). Krebs-Ringer saline (Ca 3.7 mM; K 6 mM; Mg 1.2 mM) with dextran 3.6% (M.W. 70,000, Pharmacia), bubbled with 95% O_2 to 5% CO_2 and maintained at 37° C, was delivered as pulsatile flow at constant rate (usually 8 ml/min) by a roller pump. Perfusion pressure was monitored by a transducer and Devices M2 recorder.

Mean renal perfusion pressure, initially high (range 150-250 mmHg), fell rapidly to a steady lower level (range 50-100 mmHg) within 5 min of

commencing perfusion. Experiments were begun when perfusion pressure had stabilized and timed collections of perfusate were obtained.

All pharmacological agents were infused into the arterial cannula at 0.04 ml/minute. Doses are expressed per g wet kidney weight (non-perfused kidney). Asp(NH_2)′-angiotensin II (Ciba) was diluted in barbitone buffer (0.05 M sodium barbitone, 0.02% neomycin sulphate, 0.4% human albumin) pH 8.6, and isoprenaline (isopropyl noradrenaline) was diluted in 0.9% w/v NaCl solution (saline).

Renin assay

Timed collections of perfusate were dialysed successively to pH 4.5 and pH 7.5 over 48 h at 4°C against phosphate buffers containing disodium ethylenediamine tetraacetate (EDTA), as for plasma renin activity determination by the method of Skinner (1967). After standardization of volumes, the samples were incubated at 37°C with nephrectomized rat plasma (treated by dialysis, as above) as renin substrate. Gentamycin sulphate (10 μg/ml) was added to all samples. The samples were then heated at 85°C for 10 min to terminate further reaction. After centrifugation, the supernatants were assayed without extraction for angiotensin I by radioimmunoassay (Boyd, Adamson, Fitz & Peart, 1969) using asp′-ile^5-angiotensin I (Schwartz biomedical) as assay standard. All samples from one experiment were processed and assayed at the same time. Survival of added angiotensin I was greater than 90% over the incubation period.

Renin concentration is expressed in nanogram equivalents of asp^1-ile^5-angiotensin I, generated per ml of perfusate and per 24 h incubation. Since the pump maintained constant flow rates, renin values are expressed as renin concentration without conversion to secretion rates.

Results

After perfusion pressure had stabilized, which occurred usually within 5 min after starting perfusion, a control collection for renin determination was obtained. Angiotensin was then infused, first alone and subsequently together with isoprenaline. The duration of infusions and the timing of the collections are indicated in Figure 1. The dose of isoprenaline was 0.01 μg min^{-1} g^{-1} and of angiotensin 6 ng min^{-1} g^{-1} unless otherwise stated.

In the presence of calcium in the perfusion fluid (3.7 mM), the infusion of angiotensin resulted in renal vasoconstriction with a consequent rise in renal perfusion pressure (Table 1). This was restored to control levels by the addition of isoprenaline. Renin concentration, which was reduced significantly by the infusion of angiotensin alone (Table 1), increased slowly to a level insignificantly above that of the controls at the end of the period of 9 min during which it was observed. This is in contrast with the increase in renin concentration observed when isoprenaline is infused without angiotensin (Table 3). These observations agree with our previous findings that angiotensin II suppresses isoprenaline-stimulated renin secretion.

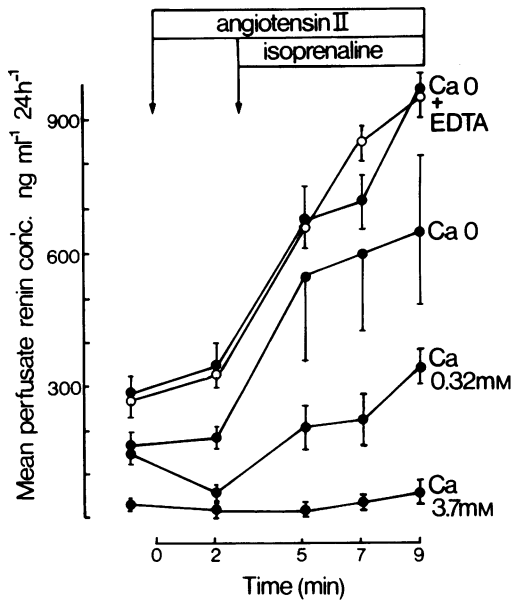

Fig. 1 The effect of angiotensin II on basal and isoprenaline-stimulated renin secretion in the presence of calcium (3.7 mM and 0.32 mM) and in the absence of calcium (+EDTA). Angiotensin, (6 ng min^{-1} g^{-1})—● and (36 ng min^{-1} g^{-1})—●, was started at 0 min and isoprenaline (0.01 μg min^{-1} g^{-1}) at 3 minutes.

When the calcium concentration was reduced from 3.7 mM to 0.32 mM, the vasoconstrictor response to angiotensin was completely abolished and the mean perfusion pressure during the infusion of angiotensin (88 ± 20 mmHg) was not significantly different from the control (80 ± 19 mmHg) ($P > 0.05$). Similarly, the mean perfusion pressure during angiotensin did not differ from the control level when calcium was excluded (91 ± 14 and 82 ± 15 mmHg, $P > 0.05$), and when EDTA (25 mM) was also added (112 ± 19 and 112 ± 19 mmHg, $P > 0.05$).

As shown in Fig. 1, the mean control renin concentration was considerably higher when

calcium was reduced (0.32 mM) or completely excluded from the perfusion fluid. Compared with the control level in the presence of 3.7 mM calcium, the differences are highly significant in each case ($P < 0.001$) (Tables 1 & 2). When the calcium concentration was reduced to 0.32 mM the inhibitory effect of angiotensin on basal renin, observed at the higher calcium level, remained in evidence (Fig. 1, Table 2). The suppression of isoprenaline-stimulated renin secretion was also still apparent, although a significant increase above control level occurred in the last collection (9 min, Table 2).

In the complete absence of calcium, suppression of basal renin secretion by angiotensin was no longer observed and a significant increase in renin concentration was seen in all collections following the infusion of isoprenaline (Fig. 1, Table 2). The addition of EDTA (25 mM) to the calcium-free perfusion fluid resulted in a further increase in the mean basal renin level, from 150 ± 22 to 227 ± 29 ng ml^{-1} 24 h^{-1} ($P < 0.01$) (Table 2). As observed without EDTA, basal renin was not suppressed by angiotensin and a prompt increase in renin concentration followed the infusion of isoprenaline.

In six experiments with calcium-free perfusion and EDTA, but without angiotensin or isoprenaline infusion, there was a further gradual increase in renin concentration over basal levels with time (160 ±40 to 346 ± 56 ng ml^{-1} 24 h^{-1} at 9 minutes). However, the mean difference between the basal level and the 9 min level (186 ± 44) remained considerably less than the corresponding difference when isoprenaline and angiotensin were present (692 ± 66) (Table 2).

In three experiments with calcium-free perfusion and added EDTA, increasing the dose of angiotensin from 6 to 36 ng min^{-1} g^{-1} did not prevent the renin response to isoprenaline (Figure 1).

Table 3 compares the effect of isoprenaline on renin concentration with calcium present (3.7 mM) and with calcium excluded from the perfusion fluid (with added EDTA, 25 mM). Compared with control levels, the mean increase in

Table 1 Effect of asp(NH_2)^1-val^5-angiotensin II alone and combined with isoprenaline on perfusate renin concentration and renal perfusion pressure in the presence of calcium (3.7 mM) (n = 8).

	Control	*Angiotensin*		*Angiotensin + Isoprenaline*	
	0 min	*2 min*	*5 min*	*7 min*	*9 min*
Mean perfusate renin concentration (± s.e. mean) (ng ml^{-1} 24 h^{-1})	22 ± 3	13 ± 1*	15 ± 2*	27 ± 9^{NS}	40 ± 15^{NS}
Mean renal perfusion pressure (± s.e. mean) (mmHg)	131 ± 25	190 ± 25***	154 ± 29*	139 ± 28^{NS}	134 ± 27^{NS}

Significance of difference from controls (paired Student's t test):
NS $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 2 Effect of asp(NH_2)^1-val^5-angiotensin II alone and combined with isoprenaline on perfusate renin concentration when calcium is reduced (0.32 mM) or absent.

	Control	*Angiotensin*		*Angiotensin + Isoprenaline*	
	0 min	*2 min*	*5 min*	*7 min*	*9 min*
Mean perfusate renin concentration (± s.e. mean) (ng ml^{-1} 24 h^{-1})					
Ca^{++} (0.32 mM) (n = 8)	142 ± 22	40 ± 5**	207 ± 45^{NS}	215 ± 53^{NS}	340 ± 52**
Ca^{++} free (n = 8)	150 ± 22	169 ± 32^{NS}	544 ± 209*	594 ± 175*	638 ± 177*
Ca^{++} free + 2NaEDTA(25 mM) (n = 6)	277 ± 29	338 ± 66^{NS}	670 ± 79**	705 ± 46***	969 ± 81***

For significance of difference from controls see Table 1.

renin concentration is generally less in the absence of calcium, although these differences are not significant.

Discussion

The common derivation of smooth muscle and juxtaglomerular cells (Barajas & Latta, 1967), together with the present observations of correlations between renin release and smooth muscle behaviour, lead to consideration of a possible common role for calcium. It appears that the calcium necessary for vascular contraction is largely derived from extracellular sources (Somlyo & Somlyo, 1968; Sullivan & Briggs, 1968) and to only a small extent from intracellular sites (van Breemen, Farinas, Gerba & McNaughton, 1972; Keatinge, 1972). The present results indicate a dependence of smooth muscle contraction and renin inhibition induced by angiotensin on the level of extracellular calcium. Contraction is more sensitive to lowering the calcium than is renin inhibition (Table 2, Fig. 1) and it suggests that a rise of intracellular calcium due to influx causes both contraction and inhibition of renin release The recent observation, that microsomal bound calcium in vascular smooth muscle membrane is released by angiotensin (Baudouin *et al.*, 1972; Baudouin-Legros & Meyer, 1972), supports this interpretation, although the source of the calcium would be different. The observations would be consistent if membrane-bound calcium is directly related to or derived from external calcium. The increase in basal renin secretion observed during calcium-free perfusions might then be explained by a lowering of the intracellular calcium levels by efflux, as observed in other situations (Goodman, Weiss, Weinberg & Pomarantz, 1972). The action of isoprenaline, in relaxing smooth muscle and stimulating renin release, might then depend on lowering intracellular calcium levels either by increased efflux or by fixation to intracellular storage sites. When phenoxybenzamine, an α-adrenoceptor antagonist, was used to block the vasoconstrictor action of noradrenaline in this preparation (van Dongen *et al.*, 1973a), renin secretion was increased, suggesting an inhibitory effect associated with vasoconstriction. Since vasopressin has also been reported to inhibit renin release (Bunag, Page & McCubbin, 1967; Vander, 1968), this effect may be common to many vasoconstrictors, other than angiotensin, and finally determined by similar mechanisms. In this respect, it has been shown (van Dongen, *et al.*, 1973b) that the inhibition of renin secretion by angiotensin is specific for biologically active angiotensin and is not observed with inactive derivatives and analogues. The observation that renin secretion remained suppressed during angiotensin infusion, even when perfusion pressure was returned to control levels by isoprenaline, is confirmed in the present study. Furthermore, reduction of the concentration of calcium from 3.7 mM to 0.32 mM abolished the vasoconstrictor effect of angiotensin but not the inhibition of renin secretion. These findings suggest that inhibition of renin secretion is not a direct result of renal vasoconstriction but rather indicates a common calcium-dependent mechanism. These proposals clearly need verification and elaboration in the isolated juxtaglomerular cells where ionic flux and content may be concurrently determined.

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Table 3 Effect of isoprenaline on perfusate renin concentration in the presence of calcium (3.7 mM) and in the absence of calcium (with EDTA).

	Control	*Isoprenaline*		
	0 min	*3 min*	*5 min*	*7 min*
Mean perfusate renin concentration (± s.e. mean) (ng ml^{-1} 24 h^{-1})				
Ca^{++} (3.7 mM) (n = 6)	52 ± 12	286 ± 59 (234 ± 60)	466 ± 127 (414 ± 133)	500 ± 134 (448 ± 140)
Ca^{++} free + 2Na EDTA (25 mM) (n = 5)	307 ± 33	403 ± 54 (96 ± 35)	506 ± 88 (199 ± 65)	644 ± 89 (337 ± 67)

The difference between the controls in the two groups is significant ($P = < 0.001$). The values in parentheses refer to the mean increase from control levels, and neither these nor the mean values for renin concentration at the corresponding time intervals in each group are significantly different from one another.

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