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Stimulation and suppression of renin release from incubations of rat renal cortex by factors affecting calcium flux

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1 Inhibition of renin secretion from incubations of rat kidney cortex by angiotensin II (AII), ouabain and K^+ depletion, depended on the presence of external Ca^{2+}.

2 AII inhibition of isoprenaline-stimulated renin secretion was only partially dependent on external Ca^{2+}.

3 Ouabain and K^+ depletion inhibited isoprenaline-stimulated renin release but only in the presence of external Ca^{2+}. Since, in Ca^{2+}-free medium, isoprenaline stimulated renin release when the Na^+/K^+-ATPase was blocked, isoprenaline probably does not act through the Na^+/K^+-ATPase.

4 Lanthanum blocked the stimulation of renin release by isoprenaline.

5 Ethylenediamine tetra-acetic acid (EDTA) and ethyleneglycol-*bis*-(β-amino-ethyl ether) N,N'-tetra-acetic acid (EGTA) increased renin secretion to a similar degree in Ca^{2+}- and Mg^{2+}-free buffer. When Mg^{2+} was present the effect of EGTA, but not EDTA, was considerably reduced.

6 Verapamil reduced the fall in basal renin secretion in normal but not Ca^{2+}-free buffer. Verapamil did not block the inhibitory effects of AII or ouabain and did not alter the stimulation of renin secretion by isoprenaline.

7 Bay K 8644 inhibited renin secretion from cortex incubated in medium containing 15 mM K^+ and this was dependent on extracellular Ca^{2+}. In normal buffer (5.9 mM K^+) Bay K 8644 increased renin secretion.

Introduction

The hypothesis that renin secretion is inversely proportional to the intracellular Ca^{2+} concentration in the juxtaglomerular (JG) cells came from studies on the isolated perfused kidney. The inhibitory effect of angiotensin II (AII) on basal and isoprenaline-stimulated renin secretion was shown to be dependent on the presence of Ca^{2+} in the perfusion fluid (Van Dongen & Peart, 1974). Similarly, inhibition of renin secretion from incubations of rat renal cortex by various agents is dependent on the presence of extracellular Ca^{2+} (Churchill & Churchill, 1980a; 1982a). These findings suggest that inhibition of renin secretion depends on influx of Ca^{2+} leading to an increase in intracellular Ca^{2+} in JG cells. However, the mechanism by which AII increases intracellular Ca^{2+} in JG cells is unknown. Which channels are involved is unclear since the effects of Ca^{2+} antagonists, which block potential dependent channels, on AII inhibition of renin release from kidney slices are equivocal (Churchill *et al.*, 1981; Naftilan & Oparil, 1982; Park *et al.*, 1981). AII may also release Ca^{2+} from intracellular stores as has been reported in smooth muscle (Baudouin *et al.*, 1972) from which JG cells are thought to be derived (Barajas & Latta, 1967).

Stimulation of renin secretion by isoprenaline is thought to result from a reduction in intracellular Ca^{2+} since in kidney slices, manoeuvres which cause Ca^{2+} influx antagonize the effect of isoprenaline (Churchill & Churchill, 1982b). By analogy with smooth muscle, it has been suggested that isoprenaline may stimulate the Na^+/K^+-ATPase, thereby increasing Ca^{2+} efflux via Na^+-Ca^{2+} exchange (Fray, 1980; Churchill & Churchill, 1982a). It is unlikely that isoprenaline alters Ca^{2+} flux through potential-dependent channels as Ca^{2+} antagonists have no effect on isoprenaline stimulated renin release either from kidney slices (Naftilan & Oparil, 1982) or from the isolated perfused kidney (Logan & Chatzilias, 1980). Renin secretion can also be stimulated by ethylenediamine tetra-acetic acid (EDTA) and ethylene-

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glycol-*bis*-(β-amino-ethyl ether) N,N'-tetra-acetic acid (EGTA) as demonstrated by Peart *et al.* (1977) in the perfused kidney and is further evidence that a fall in intracellular Ca^{2+} stimulates renin release. However, the haemodynamic changes that occur in the isolated perfused kidney may have influenced renin secretion.

In the present study we have used an *in vitro* rat kidney cortex preparation, in which any haemodynamic variables are absent, to examine the role of Ca^{2+} in isoprenalne stimulation and AII inhibition of renin secretion. The movement of Ca^{2+} into the cells has been stimulated by inhibition of the Na^+/K^+-ATPase with ouabain or K^+ depletion and with the Ca^{2+} agonist, Bay K 8644, and Ca^{2+} influx has been inhibited by the Ca^{2+} antagonists verapamil and nifedipine. The effect of inhibition of transmembrane Ca^{2+} flux with lanthanum and the effects of chelation of Ca^{2+} or Ca^{2+} and Mg^{2+} with EGTA and EDTA have been examined.

Methods

Kidneys were excised from male Wistar rats (180–250 g) that had been killed by stunning and cervical dislocation. The renal capsule and medulla were removed and at room temperature the cortex was cut into 2 mm cubes which were gently pressed onto a stainless steel sieve (pore size 350 μm) previously moistened with incubation medium. The cortex remaining on top of the sieve was scraped off, equal amounts were placed in twelve 4 ml plastic tubes and 2 ml of incubation medium was added. The tubes were gently vortexed and the tissue was allowed to settle, the supernatant was removed and replaced with 2 ml of medium. The tubes were flushed with 95% O_2, 5% CO_2, then capped. The tissue was incubated at 37°C and at the end of each 15 min incubation period the tubes were gently shaken, centrifuged at 100 *g* for 1 min and the supernatant was replaced with fresh incubation medium. Each set of 12 tubes containing the kidney cortex preparation took 5–10 min to prepare and was used for one experiment.

The supernatant from the first four incubation periods was discarded. From subsequent supernatants a sample was taken for the measurement of renin activity. This sample (100 μl) from the cortex incubation was incubated with rat renin substrate (50 μl) as prepared by Skinner (1967), 0.3 M EDTA (50 μl), 0.06 M 8-hydroxyquinoline (50 μl) and 25 mM Tris buffer pH 7.0 (750 μl). After 40 min incubation at 37°C the reaction was stopped by boiling for 2 min. The samples were centrifuged to remove coagulated proteins and the angiotensin I (AI) generated was measured by radioimmunoassay (Boyd *et al.*, 1977). The intra-assay variation was 4.3% and the inter-assay variation was 7.2%.

The stability of renin in the kidney cortex incubations was assessed by measuring the recovery of partially purified rat renin (Sagnella *et al.*, 1980) added to the incubation tubes. Amounts of partially purified renin that generated 0.26 ± 0.006, 0.52 ± 0.005 or 0.98 ± 0.018 pmol AI $min^{-1} ml^{-1}$ ($n = 6$) were incubated in the presence or absence of cortex for 15 min at 37°C and the renin activity was measured in the supernatants. The renin activity due to the added renin incubated in the presence of tissue was expressed as a percentage of the renin measured in the tubes to which renin was added but no tissue was present.

The effectiveness of the inhibitors in the incubation with renin substrate in preventing the degradation of AI by enzymes which may be present in the supernatant from the tissue incubations was examined. The recovery of AI incubated with the supernatants from tissue incubations in the presence of EDTA and 8-hydroxy-quinoline but in the absence of renin substrate was assessed.

The composition of the incubation medium was as follows (mM): NaCl 118.5, $NaHCO_3$ 24.9, KCl 5.9, $CaCl_2$ 2.54, NaH_2PO_4 1.18, $MgSO_4$ 1.18, glucose 10 and bovine serum albumin 0.5% (Fraction V, Sigma). The medium was gassed with 95% O_2 : 5% CO_2, and the final pH was 7.4. When KCl was omitted from the buffer it was replaced with equivalent amounts of NaCl. In Ca^{2+} depletion experiments the tissue was incubated in normal buffer for the first preincubation period and then in Ca^{2+}-free buffer for all subsequent incubations. $CaCl_2$ and $MgCl_2$ were omitted without replacement.

All test substances except ouabain were dissolved at 100 times the final concentration and 20 μl was added to the incubation tubes containing 2 ml medium. EDTA disodium salt (BDH) and EGTA (Sigma) were dissolved in 0.9% NaCl. (−)-Isoprenaline (+)-bitartrate (Sigma) was dissolved in 0.9% NaCl containing ascorbic acid (Sigma) to give a final concentration of ascorbic acid of 6×10^{-5} M in the incubation tubes. This concentration of ascorbic acid had no effect on renin release. AII (Ciba) was dissolved in 0.9% saline containing 0.5% bovine serum albumin. Verapamil (Abbot) was dissolved in 0.9% saline. Nifedipine (Bayer) and Bay K 8644 (Bayer) were each dissolved in ethanol at a concentration of 3×10^{-3} M and then further diluted in 0.9% saline. Equivalent amounts of ethanol were added to the controls and had no effect on renin secretion. All procedures in which nifedipine or Bay K 8644 were used were performed in the dark under sodium light due to the light sensitivity of these compounds. Ouabain octahydrate (Sigma) was dissolved in 0.9% saline at 20 times the required final concentration and 100 μl was added to 1.9 ml incubation medium.

Renin secretion is expressed as the percentage change from the baseline level measured in the first

incubation period and the results from these experiments are presented as the mean ± s.e.mean of six observations. Unpaired Student's t test was used to assess the statistical significance of differences due to treatment.

Results

The percentage of the tissue renin content secreted in the first 15 min incubation period was 1.36 ± 0.09% ($n = 6$) and by the eighth incubation period was 0.75 ± 0.02%. This low basal secretion together with the finding that inhibitors of secretion reduced it to less than 0.1% of the tissue content per hour suggests that there is no significant passive leak of renin from the tissue. The dry weight of the kidney cortex was 3.2 ± 0.12 mg per tube and the renin secreted in a 15 min incubation period generated 0.15 ± 0.006 pmol AI $min^{-1} mg^{-1}$ dry weight tissue ($n = 8$).

The percentage recoveries of renin, with activities of 0.26, 0.52 and 0.99 pmol AI $min^{-1} ml^{-1}$, incubated for 15 min at 37°C in the presence of kidney cortex were 124, 106 and 102% respectively. Thus there is no degradation, metabolism or inactivation of the renin secreted by the tissue during the incubation. The stability of AI in the substrate incubation was demonstrated by the finding that the concentrations of AI, after 40 min incubation at 37°C with EDTA and 8-hydroxy-quinoline in the presence and absence of supernatant from the kidney cortex incubation, were 0.217 ± 0.003 pmol ml^{-1} and 0.222 ± 0.003 pmol ml^{-1} respectively ($n = 6$). This complete recovery of AI demonstrates that any converting enzyme or angiotensinases that may be present in the supernatant from the tissue incubation are effectively blocked by the inhibitors present.

Microscopical examination of pieces of cortex from kidneys perfused with a suspension of charcoal particles of approximately 5 μm diameter demonstrated that the tissue used in the incubations contained afferent arterioles and glomeruli.

Effect of AII on basal and isoprenaline-stimulated renin release in normal and Ca^{2+}-free medium

Basal renin secretion from kidney cortex incubated in normal medium (2.54 mM Ca^{2+}) fell with time (Figure 1a). AII (10^{-5} M) significantly inhibited renin secretion in normal medium so that by the fifth

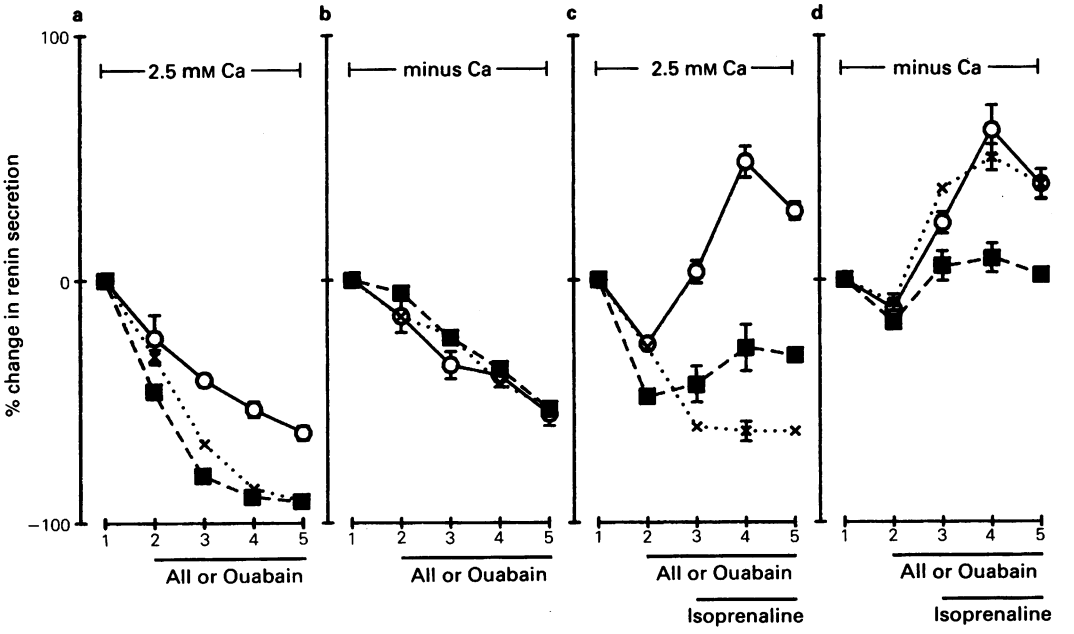

Figure 1 Effect of angiotensin II (AII, 10^{-5} M) or ouabain (5×10^{-4} M) on basal (a and b) and isoprenaline (10^{-6} M)-stimulated (c and d) renin release from rat kidney cortex incubated in normal 2.5 mM Ca^{2+} medium (a and c) or Ca^{2+}-free medium (b and d). (a and b) Control (○); AII 10^{-5} M (■); ouabain (5×10^{-4} M) (×). (c and d) Isoprenaline (10^{-6} M) (○); AII plus isoprenaline (■); ouabain plus isoprenaline (×). Renin secretion in each 15 min incubation period is expressed as the percentage change from the baseline level. Means are shown with s.e.mean indicated by vertical line except where it is less than the symbol size.

incubation period, the last four in the presence of AII, secretion had fallen by 91.8 ± 1.6% of the baseline value compared with a fall of 62.5 ± 3.0% in the controls ($P < 0.001$) (Figure 1a). However, in the absence of Ca^{2+} the inhibitory effect of AII on basal renin was abolished (Figure 1b).

Renin secretion from tissue incubated in normal medium containing 2.54 mM Ca^{2+} was significantly increased ($P < 0.001$) by isoprenaline (10^{-6} M) and reached a peak of 48.3 ± 6.4% above baseline in the second incubation period that isoprenaline was added (Figure 1c) compared with a fall of 52.7 ± 3.2% over the same time period in the controls (Figure 1a). Addition of AII (10^{-5} M) for one incubation period before and then together with isoprenaline significantly reduced the stimulation of renin secretion by isoprenaline from cortex incubated in normal medium ($P < 0.001$) (Figure 1c).

Addition of isoprenaline (10^{-6} M) to kidney cortex incubated in Ca^{2+}-free buffer resulted in an increase in renin secretion to 61.3 ± 10.2% above baseline (Figure 1d) compared with a fall to 39.0 ± 4.8% below baseline in the controls (Figure 1b). In Ca^{2+}-free medium AII (10^{-5} M) still significantly reduced the increase in renin secretion in response to isoprenaline ($P < 0.01$) (Figure 1d), although compared with normal medium the inhibitory effect was reduced.

The absolute values of renin activity in the supernatant from the first incubation period are shown in Table 1. Each series of 6 tubes consists of three pairs of tubes containing tissue from three rats. The renin levels and variability were similar in the remaining experiments and are therefore not shown. The variation in the amount of renin in the tubes is due to differences between rats and the unequal amounts of tissue in any series of tubes. However, the percentage changes after any manoeuvre were similar, demonstrating that the results are not influenced by the amount of renin present at the start of an experiment.

Effect of ouabain on basal and isoprenaline stimulated renin secretion in normal and Ca^{2+}-free medium

Addition of ouabain (5×10^{-4} M) to cortex incubated in normal medium significantly inhibited renin secretion so that by the fifth incubation period it had fallen by 91.0 ± 1.1% of the baseline value compared with a fall of 62.5 ± 3.0% in the control tubes ($P < 0.001$) (Figure 1a). In contrast, ouabain did not inhibit basal renin secretion from tissue incubated in Ca^{2+}-free medium (Figure 1b).

Addition of ouabain (5×10^{-4} M) to cortex incubated in normal medium for one incubation period before and then subsequently with isoprenaline significantly inhibited the stimulation of renin secretion by isoprenaline ($P < 0.001$) (Figure 1c). In Ca^{2+}-free buffer the inhibitory effect of ouabain on isoprenaline-stimulated renin release was completely abolished (Figure 1d).

Effect of K^+ depletion on basal and isoprenaline-stimulated renin release in normal and Ca^{2+}-free medium

Incubation of kidney cortex in K^+-free medium containing 2.54 mM Ca^{2+} inhibited basal renin secretion to 95.3 ± 0.6% below baseline after eight incubation periods in the absence of K^+ (Figure 2b), compared with a fall of 62.5 ± 3.2% in normal buffer containing 5.9 mM K^+ (Figure 2a) ($P < 0.001$). In contrast, withdrawal of K^+ from Ca^{2+}-depleted tissue resulted in an initial increase in renin secretion to 13.5 ± 2.5% above baseline (Figure 2c) compared with a fall of 31.8 ± 2.5% by the third incubation in the control tubes (Figure 2a) ($P < 0.001$). After reaching a peak in the second incubation period that K^+ was removed, renin secretion fell and by the eighth incubation period was not significantly different from the control values but at all times secretion was

Table 1 Renin activity (pmol ml^{-1} min^{-1}) in the supernatants from the six tubes in the first incubation period in each of the eight experimental series from Figure 1

	2.5 mM Ca^{2+}		*minus Ca^{2+}*		*2.5 mM Ca^{2+}*		*minus Ca^{2+}*	
	Control	*AII*	*Control*	*AII*	*Ip*	*Ip + AII*	*Ip*	*Ip + AII*
1	0.67	0.63	0.74	0.48	1.43	1.33	1.31	1.04
2	0.61	0.65	0.85	0.46	0.93	1.18	0.93	1.22
3	0.98	0.87	0.97	0.88	1.09	1.18	1.02	1.19
4	0.95	1.06	1.23	0.85	1.27	1.11	0.98	0.86
5	1.34	1.41	1.08	0.84	0.63	0.71	0.79	0.56
6	1.25	1.10	1.50	1.09	0.72	0.86	0.61	0.74
Mean	0.96	0.95	1.06	0.77	1.01	1.06	0.94	0.94
s.e.mean	0.12	0.12	0.11	0.10	0.13	0.09	0.10	0.11

AII: angiotensin II; Ip: isoprenaline.

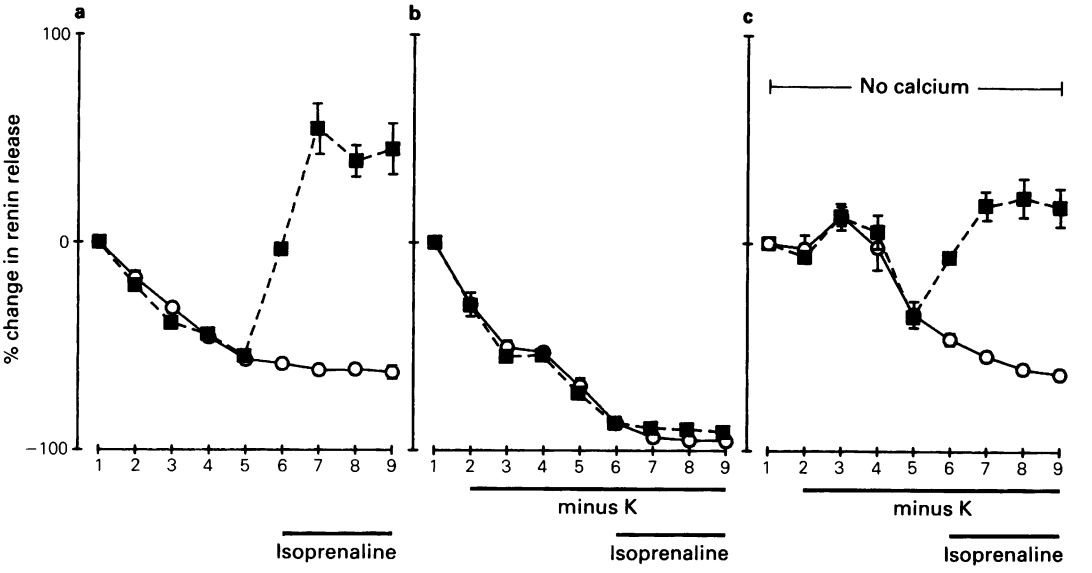

Figure 2 Basal and isoprenaline-stimulated renin secretion from rat kidney cortex incubated in normal 2.5 mM Ca^{2+}, 5.9 mM K^+ medium (a), K^+-free medium (b) or Ca^{2+}- and K^+-free medium (c). Otherwise as Figure 1. (a) Control (○); isoprenaline 10^{-6} M (■) (b and c); K^+ depletion (○); K^+ depletion plus isoprenaline (■).

significantly greater than from cortex incubated in K^+-free medium in which Ca^{2+} was present ($P < 0.001$).

Addition of isoprenaline, from the sixth to the ninth incubation periods significantly increased renin secretion from tissue incubated in normal medium ($P < 0.001$) (Figure 2a). This stimulation of renin secretion was blocked by incubation in K^+-free medium for four incubation periods before and during the addition of isoprenaline (Figure 2b). The inhibitory effect of K^+ depletion on isoprenaline-stimulated renin secretion was not present when the cortex had been incubated in Ca^{2+}-free medium during the pre and test incubations (Figure 2c).

Effect of lanthanum on isoprenaline-stimulated renin release

In the second incubation period after isoprenaline (10^{-6} M) renin secretion increased to 76.5 ± 14.2% above baseline compared with a fall to 61.8 ± 3.7% below baseline after pretreatment with $LaCl_3$ (150 μM) for one incubation period. In the controls, renin secretion fell to 9.7 ± 2.7% below baseline compared with a fall to 65.2 ± 4.4% after addition of $LaCl_3$ (150 μM) for one incubation period, which was not significantly different from the fall after $LaCl_3$ and isoprenaline.

Effect of EDTA and EGTA on renin secretion

Cortex was incubated in normal medium for the preincubations and first test incubation and thereafter in either Ca^{2+}-free or Ca^{2+}- and Mg^{2+}-free medium. In the third incubation period, different doses of EDTA or EGTA (0.1, 0.5, 1.0, 2.0 and 5.0 mM) were added. In the presence of EDTA or EGTA there was an increase in renin secretion reaching a peak after three incubation periods. The maximum increases above the controls in response to different doses of EDTA and EGTA are shown in Figure 3. The increases above the controls are expressed as a percentage of the baseline values. Addition of EDTA to tissue incubated in either Ca^{2+}- or Ca^{2+}- and Mg^{2+}-free medium caused a dose-related increase in renin secretion reaching a plateau at a dose of 2 mM EDTA (Figure 3a). A similar response was seen when EGTA was added to tissue incubated in Ca^{2+}- and Mg^{2+}-free medium (Figure 3b). However, addition of EGTA to tissue incubated in Ca^{2+}-free medium resulted in a reduced response with an increase in renin secretion of 80.7 ± 22.7% compared with 190.2 ± 18.7% in Ca^{2+}- and Mg^{2+}-free medium (Figure 3b).

Effect of verapamil on renin secretion

Verapamil (5×10^{-6} M) reduced the fall in basal renin

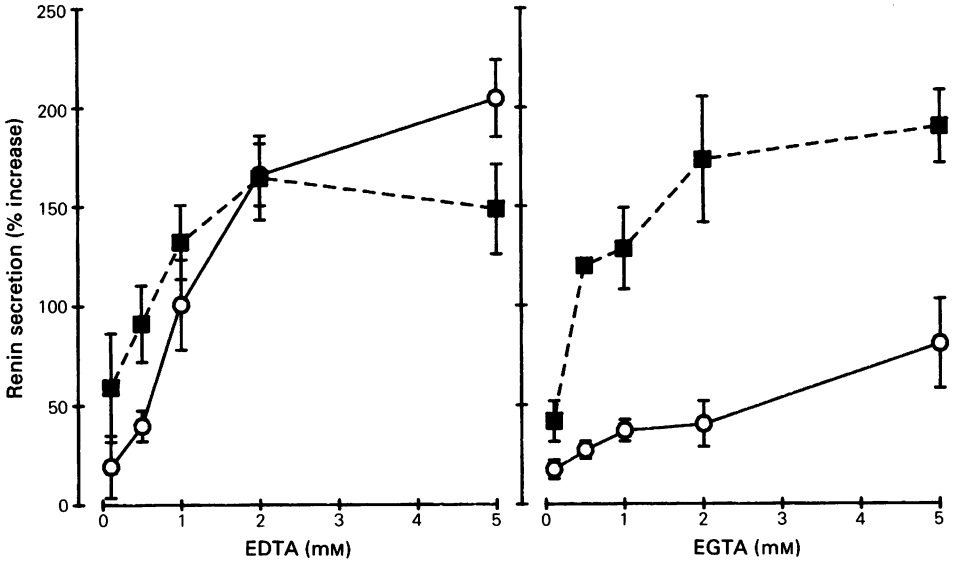

Figure 3 Effect of EDTA (a) and EGTA (b) on renin secretion from kidney cortex incubated in either Ca^{2+}-free (○) or Ca^{2+}- and Mg^{2+}-free (■) medium. The results are expressed as the maximum increase above the controls as a percentage of the values in the first incubation period vs. the dose of EDTA or EGTA.

secretion from 57.7 ± 2.0% to 13.5 ± 4.3% of the baseline value ($P < 0.001$) (Figure 4a). Addition of verapamil (5×10^{-6} M) to cortex incubated in Ca^{2+}-free buffer did not alter renin release (Figure 4b).

Addition of AII (10^{-5} M) from the third incubation period significantly increased the fall in renin secretion from 57.7 ± 2.0% in the controls (Figure 4a) to 86.0 ± 2.6% below baseline ($P < 0.001$) (Figure 4c). Renin secretion from tissue incubated in the presence of verapamil for one incubation period before and subsequently with AII was reduced to 73.5 ± 3.9% below baseline (Figure 4c) which was significantly below the levels in the presence of verapamil alone (Figure 4a) ($P < 0.001$). In the presence of verapamil, AII reduced renin secretion to 56.5 ± 15.9% below the levels when verapamil was given alone compared with a fall of 26.2 ± 8.8% in the absence of verapamil. Ouabain (5×10^{-4} M), added from the third incubation period, reduced renin secretion to 79.3 ± 5.5% below baseline which was similar to the fall of 80.8 ± 3.3% when ouabain was added in the presence of verapamil (Figure 4d).

Isoprenaline (10^{-6} M) added from the second incubation period significantly increased renin secretion to a peak value of 48.7 ± 8.8% above baseline by the fourth incubation period ($P < 0.001$) (Figure 4e). In the presence of verapamil, renin secretion in response to isoprenaline increased to 80.3 ± 8.1% above baseline (Figure 4e). However, this difference is accounted for by the higher levels of renin secretion in the presence of verapamil compared with the controls (Figure 4a). The isoprenaline-stimulated increases above the respective controls were similar in the presence and absence of verapamil.

Effects of Bay K 8644 on renin secretion in normal K^+ and high K^+ buffer

Bay K 8644 was added from the second incubation period to tissue incubated in normal buffer (5.9 mM K^+). In the control tubes renin secretion had fallen by 60.8 ± 4.9% by the fifth incubation period. After increasing doses of Bay K 8644, the falls in renin secretion were 61.0 ± 4.3% (10^{-8} M), 57.0 ± 3.4% (10^{-7} M), 49.3 ± 5.3% (10^{-6} M) and 44.3 ± 3.0% (10^{-5} M) ($P < 0.05$).

In tissue incubated in medium containing 15 mM K^+ from the second incubation period renin release had fallen to 66.8 ± 2.8% below baseline by the fifth incubation period. Addition of Bay K 8644 inhibited renin secretion and the fall in renin secretion was increased to 70.7 ± 3.3% (10^{-8} M), 74.5 ± 4.1% (10^{-7} M), 79.2 ± 4.45% (10^{-6} M) ($P < 0.05$) and 78.2 ± 4.1% (10^{-5} M) ($P < 0.05$).

The dependence of this inhibitory effect on external Ca^{2+} was examined by comparing the effect of Bay K 8644 on basal renin release from cortex incubated in 15 mM K^+ buffer either containing 2.5 mM Ca^{2+} or no Ca^{2+}. In buffer containing 2.54 mM Ca^{2+} and 15 mM K^+, addition of Bay K 8644 (10^{-6} M) from

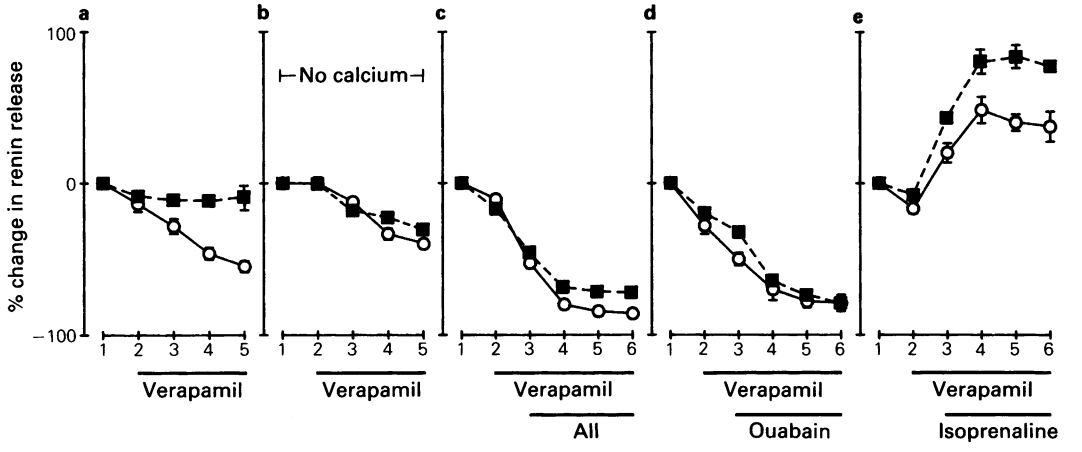

Figure 4 Effect of verapamil (5×10^{-6} M) on basal renin release in medium containing 2.5 mM Ca^{2+} (a) and in Ca^{2+}-free medium (b). The effect of verapamil on the inhibition of renin secretion by AII (10^{-5} M) (c) and ouabain (5×10^{-4} M) (d) and on the stimulation of renin secretion by isoprenaline (10^{-5} M) (e). Otherwise as Figure 1. (a and b) Control (○); verapamil (■). (c) AII (○); AII plus verapamil (■). (d) Ouabain (○); ouabain plus verapamil (■). (e) Isoprenaline (○); isoprenaline plus verapamil (■).

the third incubation period increased the fall in renin release from 64.7 ± 0.8% to 84.8 ± 0.7% of baseline by the fifth incubation period ($P < 0.001$). However, addition of Bay K 8644 (10^{-6} M) from the third incubation period to tissue incubated in Ca^{2+}-free medium containing 15 mM K^+ did not significantly reduce renin release, which fell from 68.8 ± 1.5% to 70.5 ± 1.3% of the baseline by the fifth incubation period.

Effects of Bay K 8644 on verapamil- and nifedipine-induced increases in renin secretion

Addition of Bay K 8644 from the third incubation period to tissue incubated in buffer containing 15 mM K^+ resulted in a fall in renin release to 87.0 ± 1.0% below baseline compared with a fall to 67.4 ± 2.5% in the controls ($P < 0.001$) (Figure 5a). Addition of verapamil (5×10^{-6} M) from the fourth incubation period to tissue incubated in 15 mM K^+ buffer resulted in an increase in renin secretion from 45.6 ± 0.2% below baseline to 34.0 ± 3.9% below baseline ($P < 0.05$). In the presence of Bay K 8644 (10^{-6} M) addition of verapamil increased renin secretion from 68.8 ± 3.2% to 57.0 ± 2.9% below baseline ($P < 0.05$) (Figure 5a), and there was no significant difference between the increases in the presence or absence of Bay K 8644.

In a similar experiment the effect of Bay K 8644 on the nifedipine-induced increase in renin release from tissue incubated in 15 mM K^+ medium was studied. Administration of nifedipine (10^{-5} M) from the fourth to the seventh incubation period increased renin release from 50.8 ± 4.4% to 48.2 ± 4.4% below baseline compared with an increase from 63.7 ± 5.8% to 49.5 ± 4.4% below baseline in the presence of Bay K 8644 (Figure 5b), and these increases were not significantly different.

Effect of Bay K 8644 on isoprenaline-stimulated renin secretion

In medium containing 15 mM K^+, addition of isoprenaline (10^{-6} M) from the fourth incubation period stimulated renin release to 3.8 ± 1.2% above the baseline level compared with a fall to 74.5 ± 1.2% below baseline in the controls ($P < 0.001$). Addition of Bay K 8644 (10^{-6} M) for one incubation before and subsequently with isoprenaline did not significantly alter this response.

Discussion

Stimulation of renin secretion by β-adrenoceptor agonists in JG cells is thought to occur in response to a fall in intracellular Ca^{2+} mediated by an increase in cyclic AMP, but the mechanism by which this occurs is unknown. It has been proposed that β-agonists may stimulate Na^+/K^+-ATPase activity and indirectly increase Ca^{2+} efflux through Na^+-Ca^{2+} exchange, causing a fall in intracellular Ca^{2+} and increased renin

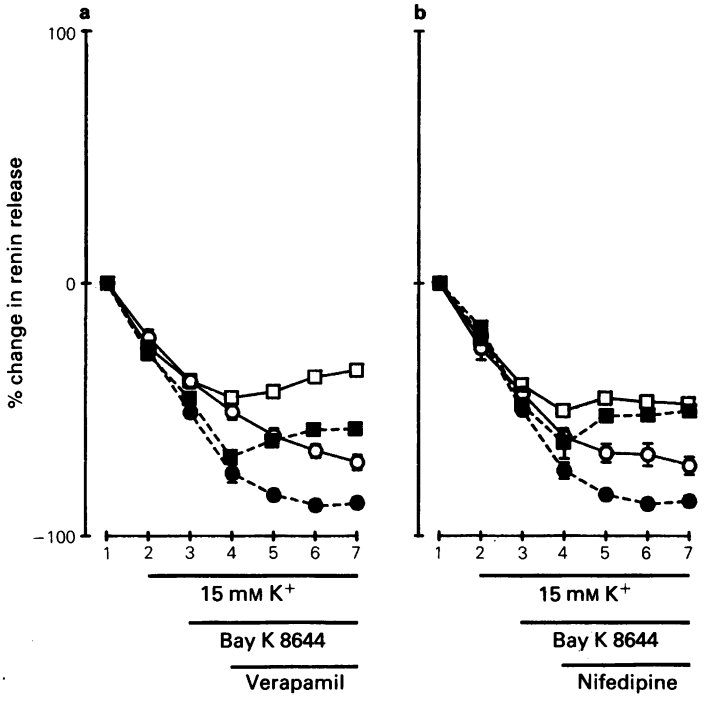

Figure 5 Effect of Bay K 8644 (10^{-6} M) on the stimulation of renin secretion by verapamil (5×10^{-6} M) (a) and by nifedipine (10^{-5} M) (b) from cortex incubated in medium containing 15 mM K^+. Otherwise as in Figure 1. (a) Control (○); Bay K 8644 (●); verapamil (□); Bay K8644 plus verapamil (■). (b) Control (○); Bay K 8644 (●); nifedipine (□); Bay K 8644 plus nifedipine (■).

secretion (Fray, 1980; Churchill & Churchill, 1982a). However, this view is not supported by the finding in the present study that in the absence of extracellular Ca^{2+}, isoprenaline stimulates renin release when the Na^+/K^+-ATPase is blocked either by K^+ depletion (Figure 2c) or with ouabain (Figure 1d). This suggests that isoprenaline is causing a fall in intracellular Ca^{2+} by some other mechanism than stimulation of the Na^+/K^+-ATPase. The similar effect of isoprenaline in normal and Ca^{2+}-free buffer (Figure 1c and d) suggests that inhibition of Ca^{2+} influx is not of major importance in the mechanism of action of isoprenaline. The finding that lanthanum, both in this preparation and in the perfused kidney (Logan *et al.*, 1977), blocks the effect of isoprenaline implies that Ca^{2+} flux across the membrane rather than sequestration of intracellular Ca^{2+} is the mechanism by which isoprenaline reduces intracellular Ca^{2+}. Isoprenaline does not appear to alter Ca^{2+} flux through potential-dependent channels as the effects of maximally stimulating doses of verapamil and isoprenaline were additive, suggesting that they act through different mechanisms. This is supported by the finding that Ca^{2+} antagonists have no effect on renin secretion either from kidney slices (Naftilan & Oparil, 1982) or from the isolated perfused kidney (Logan & Chatzilias, 1980) and the present finding that the Ca^{2+} agonist Bay K 8644 does not affect the stimulation of renin release by isoprenaline. Thus stimulation of Ca^{2+} efflux, perhaps through a Ca^{2+}-ATPase pump, is probably the dominant mechanism by which β-agonists decrease intracellular Ca^{2+} in JG cells leading to increased renin release.

The ability of AII to reduce isoprenaline-stimulated renin secretion in Ca^{2+}-free medium suggests that two mechanisms, one dependent on extracellular Ca^{2+} and the other independent, are involved. The component dependent on extracellular Ca^{2+} probably results from stimulation of Ca^{2+} influx as has been shown for AII in smooth muscle (Sullivan & Briggs, 1968). This appears to be through receptor-operated channels as verapamil, which blocks flux through potential-dependent channels, does not antagonize the inhibition of renin secretion by AII (Figure 4c). Similarly, neither diltiazem nor methoxyverapamil (D600) were found to block the inhibitory effects of AII on renin secretion from rat kidney slices (Churchill, 1980; Churchill *et al.*, 1981). In contrast, it has been reported that the effect

of AII was blocked by D600 in rat kidney slices (Naftilan & Oparil, 1982) and by verapamil in dog kidney slices (Park *et al.*, 1981) and these differences may reflect the drugs and species used. The ability of AII to inhibit isoprenaline-stimulated renin secretion from cortex incubated in Ca^{2+}-free medium could result from AII-stimulated influx of Ca^{2+} remaining in extracellular spaces. However this seems unlikely since the inhibitory effects of AII, ouabain and K^+ depletion on basal renin secretion were blocked by incubation in Ca^{2+}-free medium (Figure 1b and 2c). Furthermore the inhibition of isoprenaline stimulated renin release by ouabain (Figure 1d) and K^+ depletion (Figure 2c) were also blocked in Ca^{2+}-free medium. The finding that AII did not reduce basal renin secretion from cortex incubated in Ca^{2+}-free medium (Figure 1b) suggests that in JG cells the action of AII does not depend on the release of intracellular Ca^{2+}. AII may inhibit the action of isoprenaline by inhibition of adenyl cyclase activity as reported in membranes from several tissues including kidney (Woodcock & Johnston, 1982) and pituitary (Marie *et al.*, 1985). Pretreatment of rats with pertussis toxin, which inactivates the inhibitory protein (N_I) that mediates receptor-induced inhibition of adenyl cyclase, attenuates the inhibition of renin secretion by AII from the perfused kidney (Hackenthal *et al.*, 1985). This suggests that AII may inhibit isoprenaline stimulated adenylate cyclase activity in JG cells, reduce the fall in intracellular Ca^{2+} in response to isoprenaline and thereby inhibit stimulation of renin secretion.

Verapamil did not block the inhibition of renin release by ouabain (Figure 4d). These results agree with the report that D600 did not block the inhibitory effect on renin secretion of incubation in a low K^+ medium, which is also thought to act by inhibition of the Na^+/K^+-ATPase (Churchill & Churchill, 1980b). In view of these findings it is difficult to account for the block of the ouabain inhibition of renin release from dog kidney slices reported by Park *et al.* (1981).

Basal renin secretion was inhibited by AII, ouabain and K^+ depletion from cortex incubated in medium containing 2.54 mM Ca^{2+} but not in Ca^{2+}-free medium confirming previous reports (Van Dongen & Peart, 1974; Churchill & Churchill, 1980a; May & Peart, 1984). These studies suggest that inhibition of basal renin secretion is due to increased Ca^{2+} influx resulting in raised intracellular Ca^{2+}. Verapamil reduced the fall in basal renin secretion (Figure 4a) suggesting that there is a basal Ca^{2+} influx through potential-dependent channels, and this is supported by the finding that this effect is dependent on external Ca^{2+} (Figure 4b). Both verapamil and nifedipine reduced the fall in basal release from tissue incubated in medium containing 15 mM K^+ (Figure 5). These effects were not antagonized by the Ca^{2+} agonist Bay K 8644, which increases Ca^{2+} flux through potential-dependent channels (Schramm *et al.*, 1983), probably reflecting the high doses of the Ca^{2+} antagonists used.

Bay K 8644 added to cortex incubated in buffer containing a moderately depolarizing concentration of K^+ (15 mM) resulted in a dose-related inhibition of renin secretion. In the presence of 15 mM K^+ there is a greater probability that the Ca^{2+} channels will be in the open mode and Bay K 8644 is thought to stabilize the channels in this mode thereby increasing Ca^{2+} flux into the cell (Hess *et al.*, 1984). A similar requirement for partial depolarization in order to demonstrate the effects of Bay K 8644 has been found in rabbit aorta (Schramm *et al.*, 1983) and in perfused cat adrenal glands (García *et al.*, 1984). The observation that the inhibitory effect of Bay K 8644 in 15 mM K^+ medium was abolished if Ca^{2+} was not present in the medium is evidence that Bay K 8644 stimulates Ca^{2+} influx which leads to an increase in cytoplasmic Ca^{2+} in the JG cells, thereby inhibiting renin secretion.

In contrast Bay K 8644, in high concentrations, added to cortex incubated in normal medium stimulated renin release apparently acting as a Ca^{2+} antagonist. Previous reports have demonstrated that Bay K 8644 exhibits both agonist and antagonist properties depending on its concentration and on the membrane potential (García *et al.*, 1984) and similar effects have been described for other dihydropyridines (Hess *et al.*, 1984).

The effects of chelation of extracellular Ca^{2+} and Mg^{2+} on renin secretion were examined by the use of EDTA, which has similar affinities for Ca^{2+} and Mg^{2+}, and EGTA, which has a greater affinity for Ca^{2+} than Mg^{2+}. EDTA increased renin secretion to a similar degree either when Ca^{2+} was absent or when both Ca^{2+} and Mg^{2+} were absent. Although EGTA was as effective as EDTA in increasing renin release in the absence of Ca^{2+} and Mg^{2+}, when only Ca^{2+} was removed the increase in renin secretion was reduced by 50%, suggesting that Mg^{2+} may be substituting for Ca^{2+} and playing an inhibitory role with regard to renin secretion. These effects of EDTA and EGTA are similar to those found in the isolated perfused kidney of the rat (Peart *et al.*, 1977). The ability of EGTA and EDTA to increase renin release in Ca^{2+}-free medium suggests that they must be reducing intracellular Ca^{2+} in the JG cells. This may result from chelation of a non-exchangeable extracellular Ca^{2+} pool, removal of Ca^{2+} from membrane binding sites or to an effect on membrane polarization that alters Ca^{2+} permeability.

These studies suggest that the inhibitory effect of AII on basal renin secretion results from Ca^{2+} influx and an increase in cytoplasmic Ca^{2+}. However, this mechanism only partially accounts for the inhibition by AII of isoprenaline-stimulated renin secretion, whereas the inhibitory effects of ouabain and K^+ depletion were both blocked in the absence of extracellular Ca^{2+}. Stimulation of renin secretion by

isoprenaline is thought to result from a reduction in intracellular Ca^{2+}. The present findings show that this does not result from stimulation of Na^+/K^+-ATPase leading to Ca^{2+} efflux via Na^+-Ca^{2+} exchange and it is suggested that increased Ca^{2+} efflux, perhaps via a Ca^{2+}-ATPase, may be involved. Movement of Ca^{2+} through potential-dependent channels may influence basal renin secretion but does not appear to be involved in either the inhibition of renin release by AII or the stimulation by isoprenaline.

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