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EFFECTS OF ADRENALINE, NORADRENALINE, ISOPRENALINE AND SALBUTAMOL ON THE PRODUCTION AND RELEASE OF RENIN BY ISOLATED RENAL CORTICAL CELLS OF THE CAT

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1 Isolated renal cortical cells of the cat have been demonstrated to produce renin on incubation *in vitro*. After 2 h of incubation, without added agonist, the total amount of renin in the flask increased by a mean of 27.2%. The increase in renin content of the incubation flask was found to be present in the medium.

2 Noradrenaline (1.18×10^{-4} M) and adrenaline (1.09×10^{-4}M) added to the incubation medium stimulated renin production by 45 and 34% respectively, compared with the incubated controls. Most of the increase in renin production was present in the incubation medium.

3 Isoprenaline did not stimulate renin production. However, when added to the incubation medium at a concentration of 0.72×10^{-4}M there was a significant decrease in the cellular content and a significant increase in the medium content of renin. This increase was at least as great as that observed with adrenaline and noradrenaline.

4 Salbutamol had an effect similar to isoprenaline, i.e. it induced the release of renin into the medium without affecting production. In this respect it was about a third as potent as isoprenaline.

Introduction

A number of stimuli have been reported to alter the release of renin *in vivo*. These include changes in renal haemodynamics, changes in rate of sodium transport within the renal tubules, and the actions of catecholamines, either locally released or carried to the renin-containing cells via the circulation (cf. Vander, 1967; Davis, 1971). While the first two of these mechanisms are probably only amenable to study *in vivo*, the action of catecholamines can be studied on the isolated renal cortical cell. The technique would appear to be particularly useful in studying the direct effect of sympathomimetic substances, in isolation from other mechanisms of renin release which might be activated *in vivo* by these drugs. The method has been used by Michelakis, Caudle & Liddle (1969) in a study of the effect of adrenaline, noradrenaline and cyclic adenosine 3′,5′-monophosphate (AMP) on net renin production by dog isolated renal cortical cells *in vitro*. They observed that all of these substances stimulated renin production.

There is increasing evidence that the action of catecholamines in stimulating renin release is mediated by a β-adrenoceptor, as judged by the effect of infusion of isoprenaline (Ueda, Yasuda, Takabatake, Iizuka, Iizuka, Ihori & Sakamoto, 1970; Van Dongen, Peart & Boyd, 1973) and by blockade of the renin-releasing effect of catecholamines by propranolol (Assaykeen, Clayton, Goldfein & Ganong, 1970; Meurer, 1971; Passo, Assaykeen, Goldfein & Ganong, 1971; Coot, Johns, Macleod & Singer, 1972; Loeffler, Stockigt & Ganong, 1972). The nature of the β-adrenoceptor mediating renin release is not known, as most of the work has been done with propranolol, which is non-selective. Several recent studies in hypertensive subjects have indicated that the cardioselective drugs practolol (Esler & Nestel, 1973) and ICI 66082 (Aberg, 1974) also inhibit renin production although systematic comparisons of the relative effectiveness of these drugs in blocking renin release are not available. It would appear to be of considerable therapeutic interest if the characteristics of this β-adrenoceptor were available.

In the present study, the ability of isolated renal cortical cells of the cat to produce renin on incubation *in vitro* has been investigated and the effect of added adrenaline, noradrenaline, isoprenaline and salbutamol on the production and release of renin during incubation has been studied.

Methods

Experiments were performed on kidneys obtained from 14 male cats in the weight range 3.1-6.0 kg. One kidney was removed from each of 10 cats anaesthetized with sodium pentobarbitone (42 mg/kg i.p.). Four cats were anaesthetized with ethyl chloride/ether and these had both kidneys removed.

All glassware used in this study was siliconized (Repelcote, Hopkin & Williams), thoroughly rinsed in deionized water and sterilized overnight in an oven at 120° C.

Preparation of cortical cell suspension

Cortical cell suspensions were prepared by an adaptation of the method devised for use in the dog by Michelakis *et al.* (1969). Kidneys were removed through retroperitoneal incisions and immediately placed in ice. The renal artery was then cannulated with fine polythene tubing attached to a syringe needle, and the kidney flushed through with cold 0.9% w/v NaCl solution (saline) until the fluid emerging from the renal vein was clear (approximately 30 ml). The renal vein and ureter were clamped with a Spencer-Wells forceps and incubation medium forced through the cannula until visible in the subcapsular space (6-8 ml). The incubation medium was Minimum Essential Medium with Earle's Basic Salt Solution (Bio-cult, Glasgow), to each 100 ml of which was added 1.3 ml 200 mM glutamine (Bio-cult). This medium differs in composition from that used by Michelakis *et al* (1969) in that it contains calcium at a concentration of 1.7 mM. The Spinner modified medium used by these authors contained no calcium, which has since been reported to be a requirement for optimum renin release (Michelakis, 1971).

After decapsulation, the renal cortex was removed and chopped with a scalpel blade to yield pieces 1-3 mm in each dimension. These were then placed in 25 ml incubation medium containing 0.05% w/v collagenase (Collagenase Form II, Koch-Light) and slowly stirred with a teflon-coated magnetic stirrer at room temperature and bubbled with 95% O_2, 5% CO_2 for 75 minutes. This period of digestion was compatible with maximum tissue disaggregation, but minimal cell damage.

At the end of the digestion period the mixture was filtered through a double layer of gauze and the filtrate centrifuged at 125 *g* for 5 min at 4° C. The supernatant was removed and the cells resuspended in 5 ml fresh medium at 4° C. This procedure was repeated twice more to remove all traces of collagenase. The cells were then finally resuspended in 25 ml fresh medium if one kidney had been utilized or 35 ml when two kidneys had been prepared.

Experimental incubations

Aliquots (2.5 ml) of cell suspension were pipetted into 25 ml conical flasks with ground glass stoppers. Nine or 12 flasks were prepared, depending on the amount of suspension available. These were subdivided into groups of three. One group acted as a control and to each of the flasks in other groups one of the four drugs was added. One flask from each set of three was frozen immediately to serve as a non-incubated control, and the other two were incubated for 2 h at 37° C in a shaking water bath and bubbled with 95% O_2, 5% CO_2 at 20 min intervals.

At the end of the incubation, the contents of each flask were transferred to centrifuge tubes and centrifuged at approximately 2,300 *g* for 15 min at 4° C. After the supernatant had been removed, the cells were resuspended in 5 ml fresh medium at 4° C and homogenized with a motor-driven teflon pestle. Both supernatant and homogenate were stored at −10° C until the renin was assayed. A flow chart for incubation procedures is given in Figure 1.

Assay of renin

Renin activity was estimated according to the method of Coot *et al.* (1972) developed for the cat from an original method by Ryan, McKenzie & Lee (1968).

A 50 µl experimental sample was diluted with 4.95 ml saline; 0.5 ml of this mixture was added to 0.25 ml 40 mM disodium edetate and 0.25 ml Trasylol (FBA Pharmaceuticals). A 0.25 ml sample of this final mixture was incubated at 42° C with 0.5 ml renin substrate prepared from nephrectomized cats (Coot *et al.*, 1972), for between 40 and 48 hours. Preliminary tests indicated that angiotensinase activity was negligible under these conditions. This was confirmed for each batch of incubations.

Angiotension I generated during the incubation was assayed against standard 1-asp 5-isol angiotensin I (Schwarz Bioresearch) by the rat blood-pressure bioassay. Assay of the non-incubated controls ensured that there was no interference in the bioassay from the catecholamines added to the suspension. Because of high dilutions of the renal cell homogenate necessary for the renin assay, the catecholamine concentrations were reduced to sub-threshold levels in the bioassay.

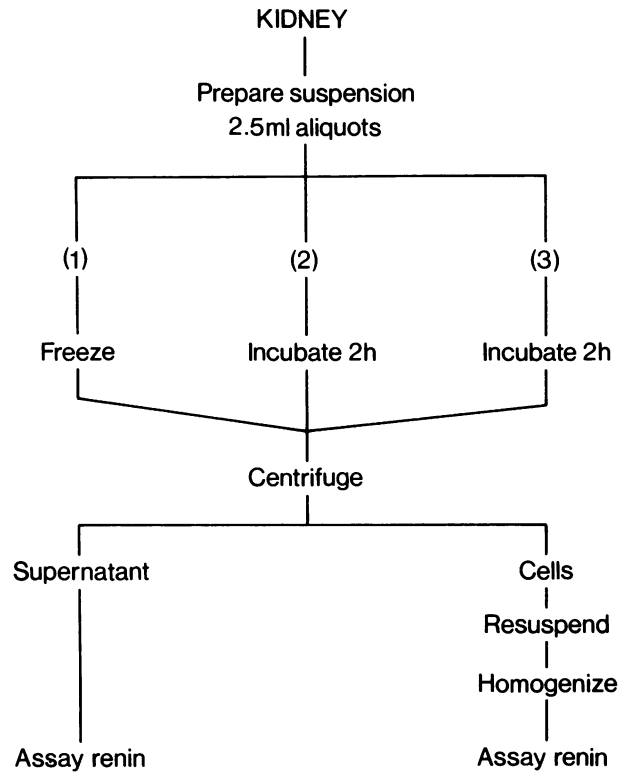

Figure 1 Procedure for the separation and incubation of renal cortical cells and preparation of the contents of the incubation flasks for renin assays.

Each sample was bracketed twice between doses of standard angiotensin I. Limits of the brackets were reduced so that the unknown could be measured at better than ± 10%.

Drugs

The following materials were used: noradrenaline (Levophed, Winthrop Laboratories); adrenaline (adrenaline tartrate, Evans Medical); isoprenaline (isoprenaline sulphate, Boots Ltd.); salbutamol (salbutamol sulphate, Allen & Hanbury, Ltd.).

Calculations

From the measured angiotensin I concentration, renin activity was expressed as ng angiotensin I generated per h incubation with substrate and per 2.5 ml suspension. The mean of the duplicates was calculated and both the incubated cell content and release were expressed as a percentage of the appropriate non-incubated control (cell content). Total renin was calculated by simple addition of the incubated cell content and release. Results were compared by Student's paired *t* test.

Results

Adrenaline and noradrenaline

These experiments were performed to indicate the sensitivity of the preparation and to confirm the action of these two catecholamines on renin secreting mechanisms in this species.

Preliminary experiments were carried out measuring total renin only, which indicated that renin production was dose-dependent at concentrations of adrenaline and noradrenaline between 0.44 and 1.90×10^{-4}M. For the present study, the doses chosen were 1.09 and 1.18×10^{-4}M as these were estimated to give 70% of the maximal response.

Table 1 gives the results obtained in eight experiments with noradrenaline and adrenaline respectively. The cell content of renin did not alter during incubation in the absence of added catecholamine nor was any significant difference observed in the presence of noradrenaline (+6.1%) or adrenaline (+1.9%) (Table 1).

Renin release increased significantly during incubation with noradrenaline. The amount

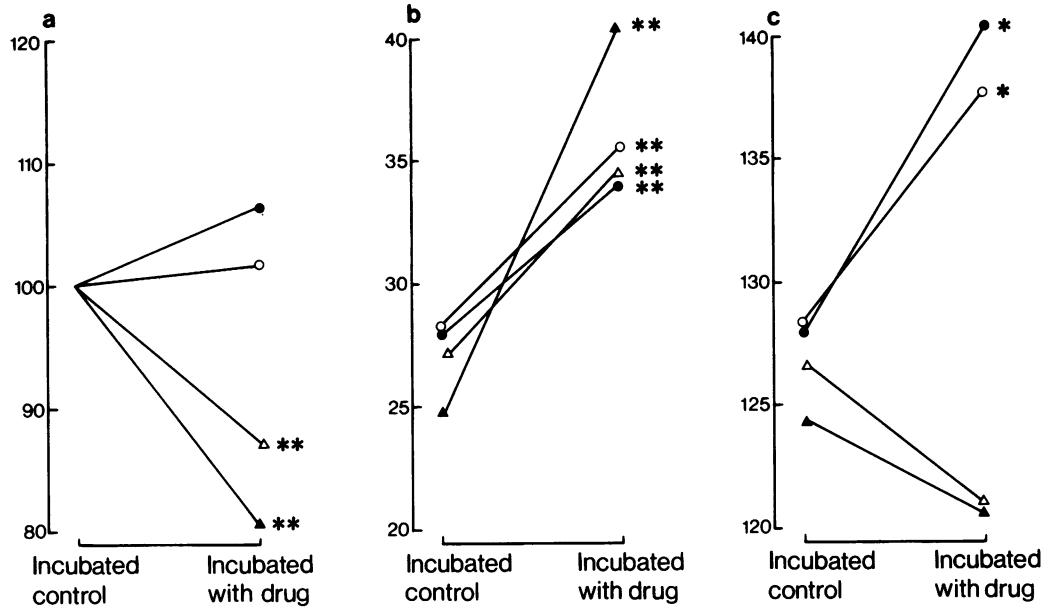

Figure 2 Effect of sympathomimetic amines on renin production and release *in vitro*; (a) cell content of renin (% of non-incubated control), (b) renin release (% of non-incubated control cell content), (c) total renin (content plus release). (●) Noradrenaline 1.18 x 10^{-4} M; (○) adrenaline 1.09 x 10^{-4} M; (▲) isoprenaline 0.72 x 10^{-4} M; (△) salbutamol 1.67 x 10^{-4} M. In all experiments $n = 8$. *$P < 0.05$; **$P < 0.01$.

released in control flasks was 27.8% (of non-incubated control values) compared with 34.3% in flasks containing noradrenaline ($P < 0.01$). A significant increase in renin release was also observed with adrenaline. The release rose from 28.3% in control flasks to 35.7% in adrenaline-containing flasks ($P < 0.01$).

When the results are expressed as the sum of cell content and the amount released, values increased from 127.8% (control) to 140.3% in the presence of noradrenaline ($P < 0.05$) and from 128.3 to 137.6% in the presence of adrenaline ($P < 0.05$).

Isoprenaline

No report has been published on the effect of isoprenaline on renal tissue *in vitro*, although it has

Table 1 Effect of noradrenaline, adrenaline, isoprenaline and salbutamol on renin production *in vitro*

Drug	*Content*		*Release*		*Total*	
	Incubated Control	*Test*	*Incubated Control*	*Test*	*Incubated Control*	*Test*
Noradrenaline (1.18 x 10^{-4}M)	100.0	106.1 ± 3.4	27.8 ± 4.6	34.3 ± 5.2**	127.8 ± 4.5	140.3 ± 6.2*
Adrenaline (1.09 x 10^{-4}M)	100.0	101.9 ± 3.1	28.3 ± 4.3	35.7 ± 4.7**	128.3 ± 4.3	137.6 ± 4.8*
Isoprenaline (0.72 x 10^{-4}M)	100.0	80.1 ± 4.5**	24.6 ± 2.95	40.5 ± 5.12**	124.6 ± 3.0	120.6 ± 3.9
Salbutamol (1.67 x 10^{-4} M)	100.0	86.1 ± 3.5**	26.9 ± 4.3	34.7 ± 4.7**	126.9 ± 4.3	120.8 ± 5.3

Results are expressed in terms of the cell content of renin in the unincubated control flasks which are designated as 100%. $n = 8$ for all drugs. Mean values with s.e. mean are given.
** Control vs drug $P < 0.01$; * Control vs drug $P < 0.05$ (Student's paired t test).

been reported to be effective in stimulating renin release *in vivo*. Table 1 shows the results of eight experiments. Isoprenaline, at a concentration of 0.72×10^{-4}M, is extremely potent in stimulating renin release. The control value was 24.6% compared with 40.5% in flasks containing isoprenaline ($P < 0.01$). The increased release seems to be at the expense of intracellular stores of renin. When isoprenaline was present during the incubation the cell content fell to 80.1% of the unincubated control value ($P < 0.01$). The total content of renin in the isoprenaline-containing flasks after incubation was 120.6% of the unincubated control value compared with 124.6% in control flasks. This decrease was not statistically significant.

Salbutamol

In order to derive more information on the nature of the β-adrenoceptor involved, the drug salbutamol, a selective β-adrenoceptor stimulant, was used. A preliminary dose-response curve showed salbutamol to be 2-3 times less potent than isoprenaline in stimulating renin release and so a concentration of 1.67×10^{-4}M was chosen to be approximately equipotent to the dose of isoprenaline used.

The results of eight experiments are presented in Table 1. It is apparent that although salbutamol is less potent than isoprenaline in stimulating renin release, the pattern of the response is similar to that obtained with isoprenaline. Cell content fell to 86.1% of the unincubated control values ($P < 0.01$). The release of renin increased from 26.9% (control) to 34.7% in the presence of salbutamol ($P < 0.01$). The total content of renin in the salbutamol containing flasks was 120.8% of the unincubated controls compared with 126.9% in the incubated control flasks. These values are not significantly different.

The results may best be compared graphically as in Figure 2. This shows the similarities between all four drugs in stimulating renin release, and shows the differences in mechanism by which this is achieved.

Discussion

The present investigation clearly indicates that isolated renal cortical cells of the cat respond to adrenaline and noradrenaline added to the incubation medium by an increase in renin production, and in this respect resemble dog renal cortical cells (Michelakis *et al.*, 1969). The dog cells may be somewhat more sensitive to the catecholamines, as in the experiments performed by Michelakis and co-workers the amount of adrenaline or noradrenaline added to the flask gave a maximum concentration of about 0.40×10^{-4}M. In the present experiments, concentrations of 1.09 and 1.18×10^{-4}M were used, although we have observed effects with concentrations as low as 0.44×10^{-4}M.

It should be stressed that these *in vitro* effects of catecholamines are produced with concentrations in the medium about 1000 times the normal plasma levels. If this relative insensitivity to the action of the catecholamines of the isolated renal cortical cells is not a methodological artefact, the implication of these results, in both species, is that circulating catecholamines do not stimulate renin release *in vivo*. It is possible, of course, that such high concentrations are achieved in the region of the nerve terminals following renal nervous activity. Such terminals have been demonstrated in close proximity to the renin-containing juxtaglomerular cells (Barajas & Latta, 1967). However, it is possible that the use of collagenase in the preparation of the cells may have destroyed membrane receptors, or that the artificial medium was not optimal for the functional activity of the cells. We have not found kidney slices, which were not exposed to the collagenase, to be any more sensitive to the catecholamines than the isolated cells (unpublished observations). De Vito, Gordon, Cabrera & Fasciolo (1970) have studied renin release by kidney slices obtained from rats and were unable to demonstrate renin release with concentrations of 0.11×10^{-4}M. However, this dose level was found to be effective in causing renin release by Rosset & Veyrat (1971) using slices from human kidneys removed for a variety of pathological conditions.

We have found that, when the cortical cells were incubated for 2 h in the absence of added catecholamine, there was an increase in the total renin content of the flask, and that the entire increase was found in the medium and never in the cells. The mean increase in renin content during incubation of all the controls of the present experiments was 27.2%. Also, when either adrenaline or noradrenaline was added to the medium the increase in total renin content observed after incubation was primarily present in the medium. The addition of noradrenaline resulted in an increase in renin production of 45% compared with incubated controls. With adrenaline the increase was 34%. The effect of the catecholamines was significant.

The effects of isoprenaline and salbutamol on renin production by renal tissue *in vitro* have not been previously reported. In our preliminary studies, in which we measured total renin content of the incubation flasks, we were unable to

demonstrate an effect of isoprenaline. However, when cell content and the amount released into the medium were estimated separately it became clear that, although a significant effect on renin production could not be demonstrated, isoprenaline had a potent effect in causing the release of cellular renin. Salbutamol, though less effective than isoprenaline, had a similar effect in stimulating the release of renin without enhancing production. Clearly, the two naturally-occurring substances affect the renin-producing cells in a different way from that of the synthetic compounds. The results are compatible with a membrane effect, by the synthetic compounds, which permits release of stored renin. The naturally-occurring substances may, on the other hand, only be stimulating renin synthesis with release following in a passive manner, depending on concentration gradients. They could also be active in inducing renin release by the same mechanism which mediated the action of the synthetic drugs. Evidence from *in vivo* studies indicates that renin release in response to renal nerve stimulation (Coot *et al.*, 1972; Loeffler *et al.*, 1972) is blocked by propranolol, indicating that the β-adrenoceptor plays an important role in the release of renin initiated by the natural transmitter.

There is little evidence regarding the biochemical events within the juxtaglomerular cells following catecholamine stimulation. Michelakis *et al.* (1969) have suggested that these substances may act by inducing the formation of cyclic AMP, as addition of this compound to the incubation medium resulted in an increase in renin production. However, in order to study the subject directly, the renin-producing cells, which constitute a very small proportion of the cells in the preparation, would have to be separated from the other cells. This could present formidable problems.

In the present study salbutamol was about one third as potent as isoprenaline in stimulating renin release *in vitro*. Comparisons of the effects of isoprenaline and salbutamol on femoral blood flow have indicated salbutamol to be one tenth as potent as isoprenaline (Cullum, Farmer, Jack & Levy, 1969), one eighth as potent (Kofi Ekue, Shanks & Zaidi, 1971) or about one third as potent (Daly, Farmer & Levy, 1971). Salbutamol has been reported to be over 100 times less potent than isoprenaline in increasing heart rate (Daly *et al.*, 1971). Current work in our laboratory, using the cardioselective blocker ICI 66082, suggests that this drug is less potent in inhibiting renin release in response to renal nerve stimulation than propranolol, although it has been reported to be equipotent in blocking the increase in heart rate due to isoprenaline (Barrett, Carter, Fitzgerald, Hull & Le Count, 1973). Although considerably more study of the renin producing and releasing mechanisms is required, these results may indicate that the receptors involved in renin release are more like the peripheral vascular than the cardiac receptors.

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