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EVIDENCE FOR THE PARTICIPATION OF β_1-ADRENOCEPTORS IN ISOPRENALINE-INDUCED RENIN RELEASE FROM RAT KIDNEY SLICES *in vitro*

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1 The inhibitory effects were studied of 4 β-adrenoceptor antagonists against renin release induced by isoprenaline (0.5 μmol/l) in rat kidney slices. Additionally the pA_2 values of these 4 drugs were measured against isoprenaline in guinea-pig isolated atria and trachea (against β_1- and β_2-adrenoceptors respectively).

2 When employed at a concentration of 2 μmol/l propranolol and atenolol significantly inhibited renin release ($P < 0.001$ and $P < 0.01$) whereas practolol and IPS 339 [*t*-butyl-amino-3 ol-2 propyl) oximino-9 fluorene] had little effect.

3 A positive correlation was shown between the degree of inhibition of renin release and the pA_2 of the antagonists at the β_1-adrenoceptors.

4 When practolol and IPS 339 were used in equipotent molar concentrations to propranolol for the β_1-adrenoceptors they inhibited renin release.

5 The results suggest that the adrenoceptor involved in the renin release induced by isoprenaline in the rat kidney is of the β_1-type.

Introduction

It is known that β-adrenoceptor agonists can cause the secretion of renin from the kidney, but the type of β-receptor involved is the subject of controversy. Since factors such as renal haemodynamics and tubular transport of sodium also affect renin secretion this problem is better studied *in vitro*.

We have shown previously (Desaulles, Forler, Velly & Schwartz, 1975) that isoprenaline induces an increase in renin release from rat kidney slices, which is dose-dependent, a plateau being obtained at a concentration of 34 nmol/l, and that this increase is blocked by propranolol. In the present study we have compared the ability of different drugs blocking β-adrenoceptors to reduce renin release evoked by isoprenaline with their pA_2 values against isoprenaline on isolated atria (β_1) and on isolated trachea (β_2).

Methods

Incubation of kidney slices

Kidneys were removed from male Wistar rats (weighing 278 ± 1 g, s.e. mean) anaesthetized with sodium pentobarbitone (50 mg/kg). After decapsulation, the kidneys were cut along the sagittal plane, the poles were ablated and the kidney half was cut manually into transverse cortico-medullary slices less than 0.5 mm thick, with an average weight of 20 mg. In each tube a single slice was incubated at 37°C in 2 ml of gassed (95% O_2:5% CO_2) 199 medium (Difco) enriched with 5% horse serum (Difco) at a pH of 7.3. Only one kidney slice was taken from each animal.

Each slice was incubated for two successive 40 min periods, (I, II) preceded by a 10 min and a 20 min preincubation, after each of which the medium was changed to rid the slices of tissue debris. No drugs were added during period I (Control), but isoprenaline (total concentration: 0.5 μmol/l) was introduced into the medium at 5 min intervals during period II, so that its possible degradation could be taken into account. Initially one third of the total dose was added 5 min after the beginning of this period, the remaining additions each being one ninth of the total dose. Some slices were exposed to β-adrenoceptor blocking drugs which were added in the appropriate concentration at the beginning of period II, 5 min

before the introduction of isoprenaline. The amounts of renin released by isoprenaline during period II were expressed as a percentage of renin released during control period I.

Measurement of renin

Physiological saline (0.3 ml) and nephrectomized rat plasma (0.5 ml) were incubated with a 0.2 ml aliquot of the medium for 2 h at 37°C, in the presence of disodium edetate (EDTA, 2.6 mM), 2,3-dimercaptopropanol (BAL, 1.6 mM) and 8-hydroxyquinoline (3.4 mM). We have observed that under these conditions there is a block of angiotensinases and of the converting enzyme. The reaction was stopped by acidification (pH 5.5) followed by 10 min in a boiling water bath. After 10 min centrifugation at 3000 rev/min and filtration on a Millipore HAWPO 1300 filter, angiotensin I was measured by radioimmunoassay according to the method of Haber, Koerner, Page, Kliman & Purnode (1969). The quantity of angiotensin I generated is proportional to the renin concentration, provided that the substrate is always in excess, as we ensured. The renin activity of the horse serum and nephrectomized rat plasma only accounted for an average of 1% of the total renin activity of the incubating medium. None of the drugs used interfered with this determination.

pA_2 determination

Guinea-pigs of either sex, weighing between 350 and 550 g, were killed with a sharp blow on the head. Their atria and tracheae were quickly excised and excess tissue was removed.

Chronotropic effect in isolated atria. (Method of Horii, Kawada, Takeda & Imai, 1974.) Atria were divided carefully into right and left halves. The right atrium, which retained spontaneous rhythm, was used to assess the chronotropic action of the drugs. The initial resting tension was set at 0.5 gram. The Krebs-Henseleit bathing solution was kept at 32 ± 1°C, and aerated with 5% CO_2 in O_2. Cumulative concentration-response curves to isoprenaline were determined first in the absence then in the presence of the blocking drug which was added 30 min before determination of the next curve.

Isolated trachea. (Method of Levy & Wilkenfeld, 1970.) The trachea was cut spirally with a sharp scalpel to produce a thin strip which was cut into two equal segments and both were placed in a bath of Krebs-Henseleit solution. The perfusion fluid contained ascorbic acid (0.1 mg/ml) and phentolamine (0.1 μg/ml). The temperature was maintained at 37°C and the solution was aerated with 5% CO_2 in O_2. An initial basal tension of 2.5 g was applied to each tracheal strip and the tissue was allowed to stand for 30 min before use. A constant level of tone was maintained by the addition of carbachol (0.1 μg/ml) to the bath 15 min before isoprenaline. Then, without washing, cumulative concentration-response curves to isoprenaline were determined first in the absence then in the presence of the blocking drug which was added 60 min before determination of the next curve.

The drugs used were: the hydrochlorides of (±)-isoprenaline (Sigma), (±)-propranolol (ICI), (+)-propranolol (ICI), (±)-practolol (ICI), and (±)-atenolol (ICI). IPS 339: hydrochloride of [(*t*-butyl-amino-3 ol-2 propyl) oximino-9 fluorene] (Imbs, Miesch, Schwartz, Velly, Leclerc, Mann, & Wermuth, 1977) was also used.

Results

pA_2 determinations

pA_2 values on isolated atria and trachea (for β_1 and β_2 adrenoceptors respectively) were as follows, results being expressed as mean ± s.d. with the number of experiments in parentheses: for (±)-propranolol 8.62 ± 0.17, (13) and 8.30 ± 0.19, (6); atenolol 7.70 ± 0.1, (6) and 6.1 ± 0.3, (12); IPS 339 7.04 ± 0.24, (10) and 9.23 ± 0.25, (25); practolol 6.85 ± 0.12, (10) and 5.13 ± 0.30 (12).

Renin release

The substance liberated by the kidney slices was identified as renin because (a) it was denatured by heating; (b) on incubation with nephrectomized rat plasma it gave a thermostable product which on immunoassay displaced labelled angiotensin I from the antibodies in exactly the same way as synthetic angiotensin I; (c) the product also produced a vasopressor response *in vivo* (unpublished observations).

Under these experimental conditions, the isoprenaline-induced renin secretion from kidney slices was an active process since no release was evoked if the experiments were conducted at 4°C. This active mechanism is a proof of the viability of the tissue under these conditions. The quantity of angiotensin I measured with radioimmunoassay was 215 ± 4 ng ml^{-1} h^{-1} (mean ± s.e. mean).

Table 1 compares the blocking activity of the 4 antagonists when used at the same concentration (2

μmol/litre). Each experiment was performed with its own control using isoprenaline alone. Propranolol (which blocks to a similar degree both β_1- and β_2-adrenoceptors) and atenolol (preferentially blocks β_1) produced a statistically significant decrease in renin release, whereas IPS 339 (β_2) and practolol (β_1) had little or no effect at this concentration. Variance analysis revealed no significant differences among the control groups ($F^3_{23} = 0.33$, $P > 0.05$) so we can suppose that the variations observed are due solely to the drugs themselves.

A possible explanation for these results is provided by Figure 1 which shows a linear regression linking the effect of the antagonists on renin release with their pA_2 values at the β_1 adrenoceptors. Variance analysis of the linear regression revealed a non-significant deviation from linearity and a significant slope test ($P < 0.001$). Moreover, the Spearmann-rank correlation coefficient (a non-parametric test) is also statistically significant ($P < 0.0005$). Thus it would seem that β-stimulated renin release takes place through the β_1 type adrenoceptor.

In accordance with this hypothesis we carried out another experiment (Table 1) with IPS 339 (76 μmol/l) and practolol (118 μmol/l) at concentrations calculated from the pA_2 values to produce the same β_1 blocking effect as 2 μmol/l propanolol. Both drugs now antagonized the effect of isoprenaline, though it should be noted that in these experiments, isoprenaline alone released proportionally more renin than in the earlier experiments (see Table 1).

None of the β-blocking agents in the concentrations employed in this study had intrinsic activity on renin release. Furthermore, we observed no correlation between the inhibition of renin release and the local anaesthetic effect; for example, 2 μmol/l (+)-propranolol (Table 1) did not inhibit renin release.

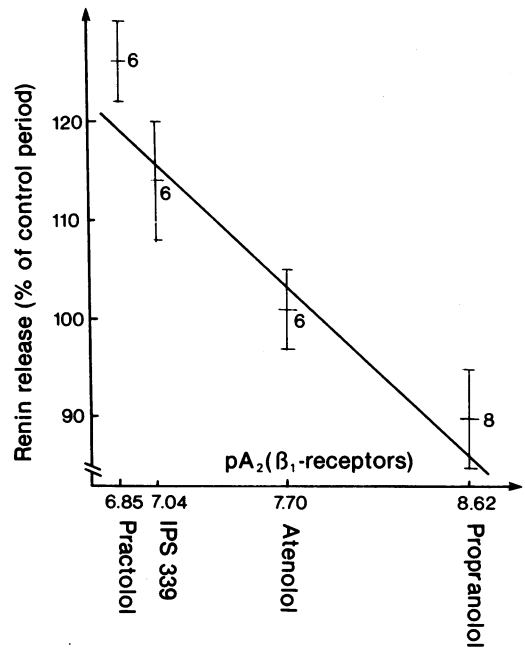

Figure 1 Isoprenaline-induced renin release in the presence of β-adrenoceptor antagonists as a function of the pA_2 for cardiac β_1-receptors (chronotropic effect). Values plotted are the mean for number of observations shown beside each point; vertical lines show s.e. means.

	Receptors	
pA_2	β_1	β_2
Propranolol	8.62	8.30
Atenolol	7.70	6.10
IPS 339	7.04	9.23
Practolol	6.85	5.13

Table 1 Effect of β-adrenoceptor-blocking drugs on renin release induced by isoprenaline

		Renin release (as % of control period)	
Drug	*Concentration (μmol/l)*	*Isoprenaline alone (0.5 μmol/l)*	*Isoprenaline + drug*
(+)-Propranolol	2	125 ± 5 (6)	128 ± 6 (6)NS
(±)-Propranolol	2	124 ± 5 (8)	90 ± 5 (8)***
Atenolol	2	130 ± 7 (6)	101 ± 4 (6)**
IPS 339	2	122 ± 9 (6)	114 ± 6 (6)NS
Practolol	2	127 ± 3 (6)	126 ± 4 (6)NS
IPS 339	76	148 ± 10 (6)	115 ± 10 (6)*
Practolol	118	147 ± 4 (6)	100 ± 4 (6)***

Results give mean values ± s.e. mean. In parentheses, the number of observations. Comparison with isoprenaline alone: Student's t test, NS not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Discussion

Our results suggest that renin release induced by isoprenaline from the rat kidney is mediated by a β_1 type adrenoceptor. It is unlikely that the β_2 receptors are involved, since IPS 339, which is 10 times more active on the trachea than propranolol, ought to inhibit renin release greatly when used in the same concentration as propranolol.

Our conclusions run counter to those of Johns, Richards & Singer (1975) in the only other *in vitro* study of which we are aware. These authors compared the effects of isoprenaline and salbutamol on renin release from isolated renal cortical cells of the cat and concluded that the receptor involved is the β_2 type. However, it is possible that the collagenase treatment which they used might modify the properties of the receptors, since isoprenaline concentrations one hundred times higher than ours were required in their experiments.

Some authors, using human subjects, conclude as we do, that the β_1-type receptor is involved: for instance, Davies, Geddes, Slater, Wiggins & Payne (1975) who compared the action of salbutamol and of isoprenaline on plasma renin activity; Attman, Aurell & Johnsson (1975) who studied the blocking effect of metoprolol and of propranolol on the increase in plasma renin induced by hydromineral depletion; Bühler, Burkart, Lütold, Küng, Marbet & Pfisterer (1975) who examined the effect on plasma renin of various β-blockers used at β_1 equivalent doses; and Bye, Johnson, Labrooy & Smith (1976) who studied the effect of practolol on the increase in plasma renin activity induced by isoprenaline and salbutamol.

However, not all investigations in man have led to this conclusion because the results of Amery, Billiet & Fagard (1974) on the effect of atenolol on plasma renin tend to indicate a β_2 type receptor. This is also suggested from experiments in the cat by Johns & Singer (1974) on the effects of propranolol and atenolol; and again, by the results of Weber, Oates & Stokes (1975) on H35/25, metoprolol and practolol in the rabbit. Whilst interspecies differences might account for certain of these divergences, it is interesting to note that, with one exception, all the studies carried out on man indicate that a β_1-type adrenoceptor is involved.

In conclusion, we suggest that the analysis of the receptor-type responsible for renin release is made easier *in vitro* because the technique avoids changes in either haemodynamics or composition of the glomerular filtrate. Furthermore, the results strongly indicate that β_1 adrenoceptors are involved in the rat kidney.

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