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CHARACTERIZATION OF BETA-ADRENOCEPTOR SUBTYPES IN RAT KIDNEY WITH NEW HIGHLY SELECTIVE β_1 BLOCKERS AND THEIR ROLE IN RENIN RELEASE

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Abstract—Highly selective beta-adrenoceptor blocking agents with a $\beta_1:\beta_2$-selectivity ratio of 0.015 to 3400 were used to characterize the β-adrenoceptors present in rat kidney and to identify those mediating renin release. The results obtained with ICYP binding to kidney membranes revealed the presence of both β_1- and β_2-adrenoceptors in a ratio of 1:1. The $pK_D\beta_1$- and $pK_D\beta_2$-values of selective β-antagonists obtained in rat kidney membranes correlated well with those found in guinea pig left ventricle (β_1) and lung (β_2), indicating that kidney receptor subtypes are pharmacologically identical with those in the ventricle and lung, respectively. In the isolated perfused rat kidney, the apparent pA_2 values of β_1-selective blockers for inhibition of isoprenaline-stimulated renin release correlated well with $pK_D\beta_1$, but not with $pK_D\beta_2$ values. These results clearly show that the β_1-adrenoceptor subtype mediates renin release in the rat kidney.

It is well established that β-adrenoceptors located on the juxtaglomerular cells in the kidney mediate renin release. The identity of the β-adrenoceptor subtype responsible for this effect vary from species to species. It has been postulated that the β_1-subtype is involved in man [1], whereas the β_2-subtype may mediate renin release in the cat [2, 3]. The β-adrenoceptor subtype mediating renin release in the rat kidney is, however, a matter of controversy. Several studies postulate that either the β_1-subtype [4, 5] or the β_2-subtype [6] is responsible for this effect. There is also a report indicating that the β-adrenoceptor mediating renin release may not discriminate between β_1- and β_2-selective agonists and antagonists [7]. Using radioligand binding to a relatively pure preparation of rat glomeruli, Summers and McPherson showed that the β-adrenoceptors present were exclusively of the β_1 type [8]. Insel [9] found both β_1- and β_2-adrenoceptor subtypes in the rat renal cortex, whereas Brodde [10] found only the β_1-subtype in the whole kidney. Engel *et al.* [20] have demonstrated by autoradiography using ICYP that only β_1-adrenoceptors are located on glomeruli of the rat kidney.

In many tissues known to possess both β-adrenoceptor subtypes, e.g. guinea-pig, human, rat and rabbit lung [11, 12] β_1- and β_2-adrenoceptors are present in the ratio of 1:4. In binding studies with these tissues, non-selective β-adrenoceptor blocking agents show a steep and monophasic displacement curve. Shallow or even biphasic curves are obtained with selective compounds according to the magnitude of their $\beta_1:\beta_2$ selectivity ratio. In the following experiments, highly β_1-selective blockers were used to characterize β-adrenoceptors in the rat kidney and the subtype responsible for renin release.

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MATERIALS AND METHODS

Chemicals. Propranolol, practolol and ICI 118-551 were kindly donated by Imperial Chemical Industries, metoprolol by Hässle, acebutolol by May & Baker, zinterol by Mead-Johnson. Other compounds, i.e. LK 203-030, 203-939, 204-155, 204-545 [13] and pindolol were synthesized in the Preclinical Research Department, Sandoz Ltd., Renin tetradecapeptide substrate was purchased from Bachem.

Preparation of rat kidney membranes. Kidney membranes were prepared as described previously [10]. Male Wistar Ivanovas rats (200–250 g body weight) were sacrificed by decapitation. Both kidneys were immediately excised, decapsulated and placed in 10 volumes of ice cold buffer consisting of 10 mM Tris–HCl, 0.154 M NaCl, pH 7.5. The kidneys were homogenized using a Polytron Type PTA 10-35 at a high speed for 20 sec. The homogenate was filtered through a gauze and centrifuged at 20,000 *g* for 20 min at 4°. The resulting pellet was resuspended in buffer, homogenized and recentrifuged. The final pellet was resuspended in buffer (about 5 ml/g of original tissue) and stored in liquid nitrogen until use.

Preparation of guinea-pig heart and lung membranes. Guinea-pigs, Sandoz breeding, 400–500 *g* body weight, were sacrificed by a blow on the head and the heart excised. Left ventricle mem-

branes were prepared according to the procedure of Ziegelhoffer *et al.* [14]. Lung membranes were obtained using the method described by Kleinstein and Glossmann [15] with modifications of Engel *et al.* [11].

Binding assay. Binding assays on membranes prepared from guinea-pig left ventricle, guinea-pig lung, and rat kidney were carried out using [^{125}Iodo]-cyanopindolol (ICYP) with slight modifications to the previously reported method [11]. The incubations were carried out in disposable polystyrene wells (Sterilin Laboratory) at 37° for 1 hour, terminated by rapid filtration and rinsing over Gelman AE glass fiber filters on Titertek Cell Harvester apparatus (Skatron A.S., Flow Laboratories, Norway). Each filter was washed for 10 seconds with about 5 ml ice cold buffer containing: 0.154 M NaCl, 10 mM Tris–HCl, 2% ethanol, pH 7.5. The radioactivity of the wet filters was measured in a Kontron gamma counter at 80% counting efficiency. (−)Propranolol 10^{-6} M was used to determine the nonspecific binding of ICYP. The equilibrium dissociation constant of ICYP and the maximal number of binding sites were calculated from the Scatchard plot [16]. Analysis of inhibition of ICYP binding by nonselective and selective β-adrenergic agents calculation of their pK_D values and receptor distribution was performed by non-linear curve fitting of the data to the function shown below using the SAAM 27 program [17].

$$\% \text{ Bound} = [1 + P(11)/P(12)] \times \left[\frac{P(2)}{1 + [P(11)/P(12)]\,[1 + T/P(13)]} + \frac{P(1) - P(2)}{1 + [P(11)/P(12)][1 + T/P(16)]}\right]$$

P(1) = Total bound (%)
P(2) = Total β_1 bound (%)
P(1) − P(2) = Total β_2 bound (%)
P(11) = Dissociation const. of the labelled ligand
P(12) = Concentration of the labelled ligand
P(13) = Dissociation const. of the cold ligand at the β_1-receptor
P(16) = Dissociation const. of the cold ligand at the β_2-receptor
T = Concentration of the cold ligand

Perfused rat kidney. Isolated rat kidneys were perfused as described by Hofbauer *et al.* [18] with small modifications. Male Wistar Ivanovas rats (200–240 g of body weight) which had access to food and water *ad lib.* prior to the experiment, were anaesthetized with Nembutal® 75 mg/kg i.p. The kidneys were perfused through the renal artery in a single pass system at a constant perfusion pressure of 90 mmHg. The perfusion medium used was modified by adding polyvinyl pyrrolidone (20 g/l) instead of Haemaccel (a standard gelatine preparation 35 g/l) and vasopressin was omitted. Renal perfusate flow and urine production were recorded continuously. All substances tested were dissolved in buffer solution and infused intra-arterially to the kidney after an equilibration period of 40 minutes. Isoprenaline was infused at concentrations ranging from 2×10^{-9} M to 10^{-6} M for 9 min at each concentration. Only one isoprenaline concentration response curve was obtained in each experiment. Beta-blockers were infused 15 min before and during isoprenaline infusion.

For the determination of renin activity (RA), venous perfusate from the last 3 min of each infusion period was collected. The determination of RA in venous effluent was performed by incubating an aliquot of perfusate for 30 min at 37°, pH 6.0 with synthetic renin tetradecapeptide substrate and inhibitors of angiotensinases. The amount of angiotensin I (ANG I) generated was determined by radioimmunoassay using a commercial kit (CIS). Renin release was expressed as ng ANG I generated/g kidney/min.

Full cumulative concentration-response curves for isoprenaline in the absence and presence of β-blockers were plotted. The isoprenaline concentration-response curves from six experiments were averaged. Each β-blocker was tested at two to four different concentrations. The apparent pA_2 values were calculated according to Furchgott [19].

RESULTS

Binding of ICYP to the rat kidney membranes

In saturation experiments specific binding of ICYP was 80% of total binding at K_D concentration. The K_D of ICYP determined by Scatchard analysis was 37.8 ± 0.81 pM and the B_{max} value 28.6 ± 4.5 fmol/mg protein (mean ± S.E., N = 6). The Scatchard plot showed in all experiments a straight line pointing out that ICYP does not descriminate under our assay conditions between β_1- and β_2-adrenoceptors.

Characterization of beta-adrenoceptors in the rat kidney by binding studies

To characterize the β-adrenoceptor subtypes present in the rat kidney, inhibition of specific ICYP binding by nonselective and by β_1 or β_2 selective agents was performed. Some representative displacement curves are shown in Fig. 1. Nonselective β-blockers (pindolol, propranolol) show a monophasic and steep displacement curve. The β_1- or β_2-selective agents with a low selectivity ratio (metoprolol, zinterol) show a monophasic but shallow displacement curve indicating an inhomogenous population of β-adrenoceptors in the tissue. Furthermore, the displacement curves obtained with compounds with a high selectivity ratio, such as 204-545 (β_1-selective) and ICI 118-551 (β_2-selective) were biphasic. The displacement curves obtained with other highly selective β_1-adrenoceptor blocking agents, i.e. LK 203-030, 203-939 and 204-155 (Table 1) were similar to that of 204-545. Data from displacement curves obtained with these compounds revealed that β_1- and β_2-adrenoceptor subtypes are present in the rat kidney in the ratio of 1:1.

The $pK_D\beta_1$ and $pK_D\beta_2$ values of selective compounds in the rat kidney were calculated and compared with corresponding values obtained in the guinea-pig left ventricle ($pK_D\beta_1$) and lung ($pK_D\beta_2$) (Table 2).

A very good correlation was obtained between $pK_D\beta_1$ values in the rat kidney and guinea-pig left

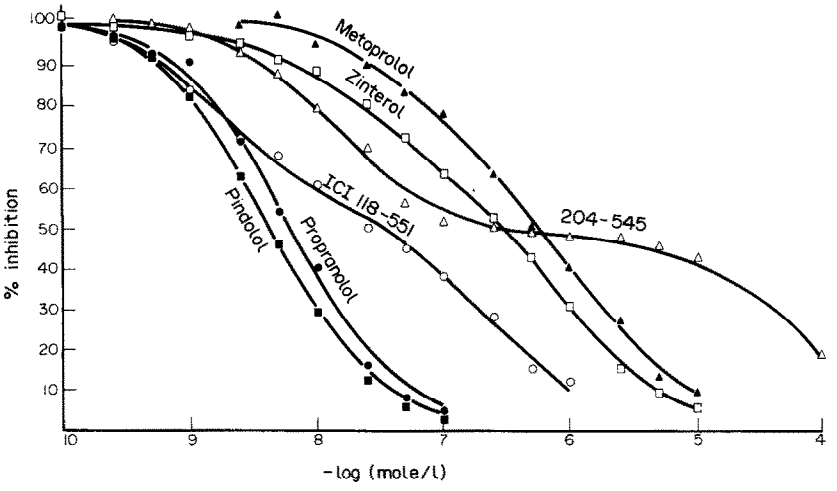


Fig. 1. Inhibition of specific ICYP binding to membranes from rat kidney by β-adrenoceptor blocking agents and zinterol. Specific binding (defined using 10^{-6} M (−)propranolol) was 80% of total binding. The K_D of ICYP determined by Scatchard analysis was 37.8 ± 0.81 pM and the B_{max} value 28.6 ± 4.5 fmol/mg protein (M ± S.E., N = 6).

ventricle (Fig. 2a) indicating that the β_1-adrenoceptors present in these two organs are identical. The same is true for the β_2-adrenoceptors present in rat kidney and guinea-pig lung (Fig. 2b).

The juxtaglomerular β-adrenoceptor subtype involved in renin release

Cumulative concentration-response curves for isoprenaline-mediated renin release in the absence and presence of β-blockers were obtained in the perfused rat kidney. The maximally effective isoprenaline concentration caused an increase of renin release of 5–10 times control values. All β-adrenoceptor blockers tested shifted the isoprenaline concentration–response curve to the right as shown for pindolol and LK 203-030 in Fig. 3. The apparent pA_2 values calculated are present in Table 2. The pA_2 values of β_1-selective blockers correlate well with the $pK_D\beta_1$ (Fig. 4a), but not with the $pK_D\beta_2$ values (Fig. 4b). These results suggest that the β_1-adrenoceptor subtype mediates renin release in the rat kidney.

DISCUSSION

The existence of two β-adrenoceptor subtypes in many tissues is well documented. The ratio between both adrenoceptor subtypes varies from one tissue to another but in general one receptor subtype predominates [11, 12].

In the rat kidney we found both β-adrenoceptor

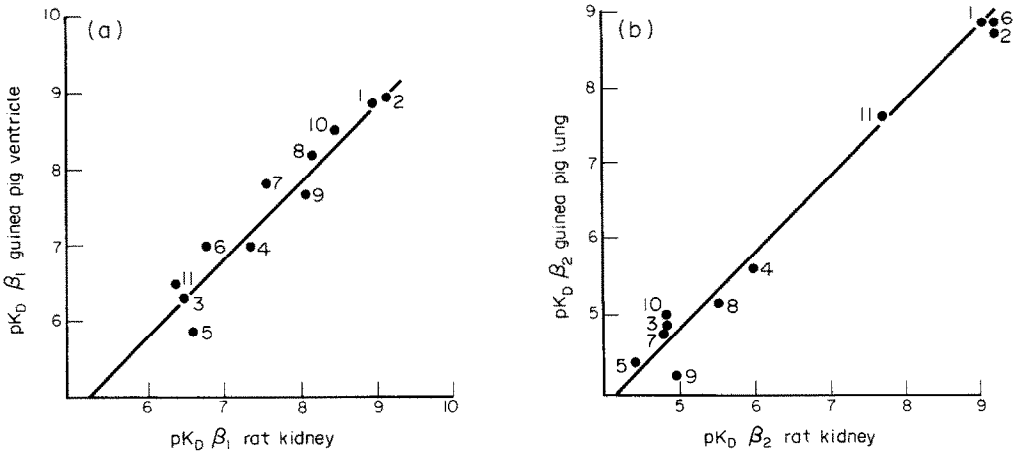
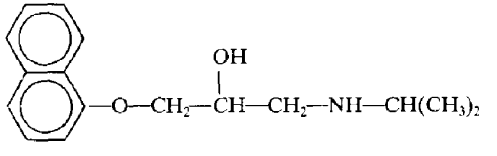
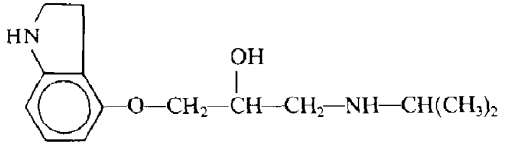
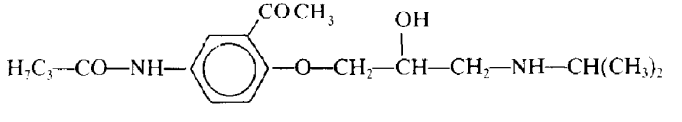
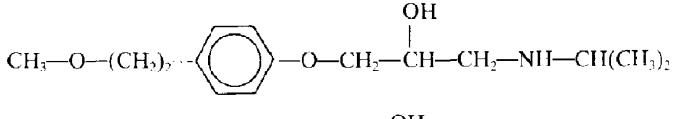
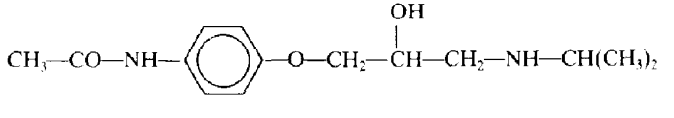


Fig. 2. (a) Correlation between $pK_D\beta_1$ values obtained in rat kidney and guinea-pig left ventricle membrane preparations. Compounds are numbered according to Table 1. $Y = 1.01x - 0.23$, $r = 0.96$; $P < 0.001$ for the slope against 0; $P > 0.4$ for the slope against 1. (b) Correlation between $pK_D\beta_2$ values obtained in rat kidney and guinea-pig lung membrane preparations. $Y = 0.97x + 0.04$, $r = 0.99$; $P < 0.001$ for the slope against 0; $P = 0.3$ for the slope against 1.

Table 1. Structures and selectivity ratios of β-adrenoceptor blocking agents and zinterol. The selectivity for β_1-adrenoceptors was calculated from guinea pig ($K_D\beta_2$ lung)/$K_D\beta_1$ ventricle).

	Substance	No.	Structure	Selectivity for β_1-adrenoceptors
β-Blockers	Propranolol	1		0.9
	Pindolol	2		1.4
	Acebutolol	3		26
	Metoprolol	4		29
	Practolol	5		33

β-Blockers	ICI 118-551	6	0.015
	LK 203-030	7	980
	203-939	8	2090
	204-155	9	2190
	204-545	10	3380
β-Agonist	Zinterol	11	0.081

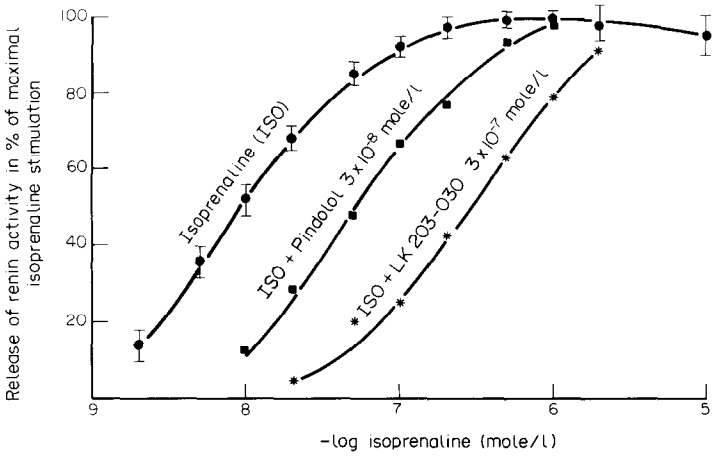


Fig. 3. Dose–response curves for isoprenaline-stimulated renin release in the absence (● mean ± S.E.M., N = 6) and presence of pindolol 3×10^{-8} mole/l (■) or LK 203-030 3×10^{-7} mole/l (★). The renin activity in the venous perfusate was measured as ANG I (ng/g kidney/min) generated after 30 min incubation with tetradecapeptide as substrate. The basal renin release was 15–20 ng ANG I/g kidney/min. Isoprenaline increased renin release maximally 5–10 times that of the basal values. The maximum isoprenaline effect in each experiment was taken as 100%.

subtypes present in equal proportions. This is at variance with the results of Brodde [10] who detected only β_1-adrenoceptors in whole kidney membranes of the rat. Failure to detect β_2-adrenoceptors may have been due to the relatively low selectivity of the β-adrenergic agents used, i.e. metoprolol and zinterol. In our experiments, the non-selective as well as the low-selective compounds show an apparently monophasic displacement curve. A biphasic displacement curve can be seen only with very selective compounds. In the case of 204-545 and other highly selective β_1-adrenoceptor blockers, a plateau separates the phases of the biphasic displacement curve, thus indicating the existence of two β-adrenoceptor subtypes in the rat kidney.

The good correlation between $pK_D\beta_1$ values of the rat kidney and guinea-pig left ventricle, and between $pK_D\beta_2$ values of the rat kidney and guinea-pig lung demonstrates that the receptor subtypes in these mammalian tissues are identical, as has been shown for many other tissues [12].

All the β-adrenoceptor blocking agents tested antagonized isoprenaline-stimulated renin release in the perfused rat kidney. The apparent pA_2 values of β_1 selective blockers correlate well with corresponding $pK_D\beta_1$ values, but not with $pK_D\beta_2$ values. These results strongly support the hypothesis that the β_1-adrenoceptor subtype mediates renin release in the rat kidney. This is in agreement with the results of Summers and McPherson [8], and Engel *et al.* [20],

Table 2. pK_D and apparent pA_2 values of several β-adrenoceptor antagonists and zinterol. Affinity constants (pK_D) on guinea-pig left ventricle, lung and rat kidney membranes were determined from the competition curves of specific ICYP binding. The apparent pA_2 values for the isoprenaline stimulated renin release were obtained in the isolated perfused rat kidney. The results represent the mean ± S.E.M. (pK_D) or the range of 2–4 separate experiments (pA_2). Number of experiments are given in parentheses.

	pK_D				
	Guinea pig		Rat kidney		
Substance	Ventricle β_1	Lung β_2	β_1	β_2	pA_2
Propranolol	8.82 ± 0.03 (13)	8.87 ± 0.03 (13)	8.99 ± 0.10 (12)	8.99 ± 0.10 (12)	8.0–8.2 (4)
Pindolol	8.94 ± 0.05 (6)	8.80 ± 0.07 (6)	9.19 ± 0.13 (6)	9.19 ± 0.13 (6)	8.0–8.3 (4)
Acebutolol	6.33 ± 0.03 (5)	4.91 ± 0.02 (6)	6.53 ± 0.12 (7)	4.83 ± 0.10 (7)	6.3–6.6 (2)
Metoprolol	7.04 ± 0.03 (5)	5.58 ± 0.03 (5)	7.41 ± 0.13 (5)	5.99 ± 0.06 (5)	7.6–7.7 (2)
Practolol	5.89 ± 0.06 (8)	4.37 ± 0.05 (6)	6.65 ± 0.15 (5)	4.43 ± 0.07 (5)	6.3–6.6 (4)
LK 203-030	7.77 ± 0.13 (5)	4.78 ± 0.06 (5)	7.61 ± 0.10 (5)	4.79 ± 0.09 (5)	7.9–8.0 (4)
203-939	8.35 ± 0.05 (5)	5.03 ± 0.04 (6)	8.21 ± 0.06 (6)	5.45 ± 0.10 (6)	7.1–7.5 (2)
204-155	7.56 ± 0.08 (7)	4.22 ± 0.05 (6)	8.01 ± 0.15 (6)	4.97 ± 0.11 (6)	7.3–7.5 (4)
204-545	8.49 ± 0.05 (5)	4.96 ± 0.07 (7)	8.54 ± 0.11 (5)	4.84 ± 0.06 (5)	8.3–8.5 (2)
ICI 118-551	7.00 ± 0.11 (7)	8.83 ± 0.15 (7)	6.77 ± 0.06 (6)	9.19 ± 0.03 (6)	
Zinterol	6.48 ± 0.03 (5)	7.57 ± 0.09 (5)	6.44 ± 0.05 (6)	7.69 ± 0.11 (6)	

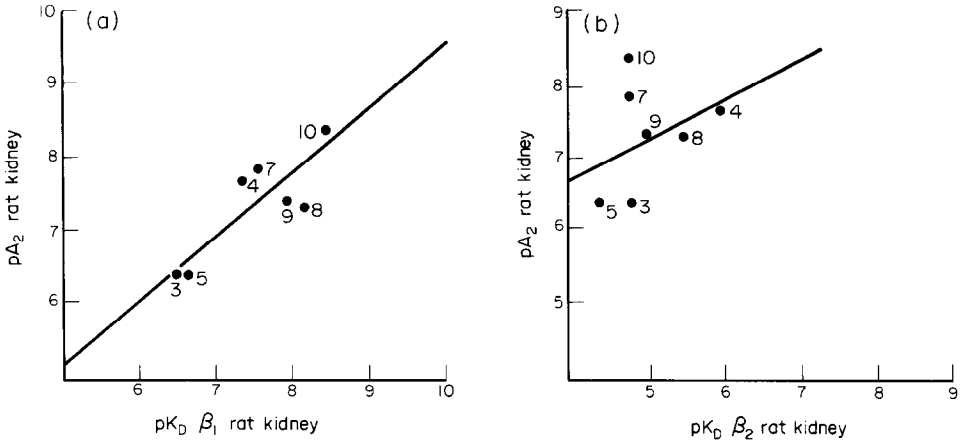


Fig. 4. (a) Correlation between $pK_D\beta_1$ values for rat kidney membranes and apparent mean pA_2 values for isoprenaline-stimulated renin release in the perfused rat kidney. $Y = 0.81x + 1.22$, $r = 0.83$; P = 0.02 for the slope against 0; P > 0.3 for the slope against 1. (b) Correlation between $pK_D\beta_2$ values for rat kidney membranes and apparent pA_2 values for isoprenaline-stimulated renin release in the perfused rat kidney. $Y = 0.46x + 5.02$, $r = 0.32$; P > 0.3 for the slope against 0; P > 0.2 for the slope against 1.

who found that only β_1-adrenoceptors are present in the rat glomeruli. Our findings are also supported by the results of Insel [9], who characterized the binding of ICYP to rat renal cortex membranes and concluded that the majority of β-adrenoceptors in the cortex are of the β_1-subtype. This can be explained by the fact that the kidney cortex is rich in glomeruli containing only the β_1-adrenoceptor subtype [5, 20].

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